A nonequilibrium thermodynamic approach to biological energy conversion systems

Michael K. Dowd
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A NONEQUILIBRIUM THERMODYNAMIC APPROACH TO BIOLOGICAL ENERGY CONVERSION SYSTEMS

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A nonequilibrium thermodynamic approach to biological energy conversion systems

by

Michael K. Dowd

A Dissertation Submitted to the Graduate Faculty in Partial Fulfillment of the Requirements for the Degree of

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### TABLE OF CONTENTS

**INTRODUCTION** ........................................ 1

**LITERATURE REVIEW AND BACKGROUND MATERIAL** ............ 3

<table>
<thead>
<tr>
<th>Linear Nonequilibrium Thermodynamics</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basic concepts</td>
<td>3</td>
</tr>
<tr>
<td>Energy conversion</td>
<td>9</td>
</tr>
<tr>
<td>Definition</td>
<td>9</td>
</tr>
<tr>
<td>Flow ratio versus force ratio</td>
<td>9</td>
</tr>
<tr>
<td>Limiting operating states</td>
<td>13</td>
</tr>
<tr>
<td>Other operating states</td>
<td>14</td>
</tr>
<tr>
<td>Effectiveness of energy conversion</td>
<td>15</td>
</tr>
</tbody>
</table>

| Biological Energy Converters | 19 |
| Skeletal Muscle | 24 |
| Skeletal muscle structure | 25 |
| Muscular contraction | 30 |
| Skeletal muscle regulatory processes | 34 |
| Skeletal muscle phosphorylation | 35 |
| Myosin reversible calcium binding | 38 |
| Skeletal muscle fiber and myosin isoenzymes | 41 |
| Muscle Nonequilibrium Thermodynamics | 43 |

**PARALLEL AND SERIES ENERGY CONVERSION SYSTEMS** .......... 48

| Simple Systems | 48 |
| Subunit dissipation function and phenomenological equations | 48 |
| Parallel-output systems | 50 |
| Limiting operating states | 53 |
| Level flow | 54 |
| Static head | 56 |
| Other operating states | 59 |
| System coupling | 59 |
| Series-output systems | 61 |
| Limiting operating states | 63 |
| Level flow | 63 |
| Static head | 63 |
| System output diagrams | 66 |
| System coupling | 66 |
| More Complex Situations | 67 |

**AN EXAMPLE - MUSCULAR CONTRACTION** ....................... 71

| Myosin Light Chain Phosphorylation | 71 |
| Mouse extensor digitorum longus (EDL) muscle | 71 |
| Phenomenological equations | 72 |
| Maximum contraction velocity | 77 |
| Isometric rate of energy use | 82 |
| Overall muscle coupling | 90 |
| Variation of cross-bridge numbers | 93 |
Recent work ................................................. 94
Rabbit psoas muscle ................................. 100
Frog sartorius muscle .............................. 103
Myosin Heavy Chain Isozyme Composition ...... 104
SUMMARY ...................................................... 108
CONCLUSIONS ............................................... 110
RECOMMENDATIONS FOR CONTINUED WORK ...... 112
BIBLIOGRAPHY ............................................... 114
ACKNOWLEDGMENTS ......................................... 125
## LIST OF TABLES

<table>
<thead>
<tr>
<th>TABLE</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>TABLE 1</td>
<td>Contraction velocity versus phosphorylation data</td>
<td>79</td>
</tr>
<tr>
<td>TABLE 2</td>
<td>Nondimensional phenomenological constants</td>
<td>81</td>
</tr>
<tr>
<td>TABLE 3</td>
<td>Energy use-tension-time integral relations</td>
<td>84</td>
</tr>
<tr>
<td>TABLE 4</td>
<td>Approximated rate of energy use data</td>
<td>88</td>
</tr>
<tr>
<td>TABLE 5</td>
<td>Constants associated with myosin-Ca$^{2+}$ binding</td>
<td>99</td>
</tr>
<tr>
<td>TABLE 6</td>
<td>Unloaded shortening speeds and isozyme content</td>
<td>105</td>
</tr>
<tr>
<td>TABLE 7</td>
<td>Comparison of maximum contraction velocity models</td>
<td>106</td>
</tr>
<tr>
<td>FIGURE</td>
<td>Description</td>
<td>PAGE</td>
</tr>
<tr>
<td>--------</td>
<td>-----------------------------------------------------------------------------</td>
<td>------</td>
</tr>
<tr>
<td>1</td>
<td>The system and environment</td>
<td>4</td>
</tr>
<tr>
<td>2</td>
<td>A two force-flow pair energy converter</td>
<td>10</td>
</tr>
<tr>
<td>3</td>
<td>Flow ratio versus force ratio</td>
<td>12</td>
</tr>
<tr>
<td>4</td>
<td>Output diagrams</td>
<td>15</td>
</tr>
<tr>
<td>5</td>
<td>Efficiency versus force ratio</td>
<td>17</td>
</tr>
<tr>
<td>6</td>
<td>The glutamine synthetase enzyme</td>
<td>22</td>
</tr>
<tr>
<td>7</td>
<td>Two-subunit two-flow energy conversion systems</td>
<td>23</td>
</tr>
<tr>
<td>8</td>
<td>Skeletal muscle</td>
<td>27</td>
</tr>
<tr>
<td>9</td>
<td>The actomyosin cross-bridge cycle</td>
<td>32</td>
</tr>
<tr>
<td>10</td>
<td>Mechanism of light chain phosphorylation</td>
<td>36</td>
</tr>
<tr>
<td>11</td>
<td>The Caplan muscle model</td>
<td>44</td>
</tr>
<tr>
<td>12</td>
<td>Parallel and series level flow</td>
<td>55</td>
</tr>
<tr>
<td>13</td>
<td>Parallel and series static head</td>
<td>57</td>
</tr>
<tr>
<td>14</td>
<td>A three subunit type parallel-series system</td>
<td>69</td>
</tr>
<tr>
<td>15</td>
<td>Maximum velocity versus relative phosphorylation</td>
<td>80</td>
</tr>
<tr>
<td>16</td>
<td>Energy use versus tension-time integral</td>
<td>85</td>
</tr>
<tr>
<td>17</td>
<td>Time variation of phosphorylation</td>
<td>87</td>
</tr>
<tr>
<td>18</td>
<td>Rate of energy use versus relative phosphorylation</td>
<td>89</td>
</tr>
<tr>
<td>19</td>
<td>Muscle coupling versus relative phosphorylation</td>
<td>92</td>
</tr>
<tr>
<td>20</td>
<td>Time dependent stimulation induced LC2 changes</td>
<td>98</td>
</tr>
<tr>
<td>21</td>
<td>Anticipated changes in contraction velocity</td>
<td>101</td>
</tr>
</tbody>
</table>
FIGURE 22. Unloaded shortening velocity versus myosin isozyme content .......................... 107
INTRODUCTION

From an engineering point of view, living organisms can be thought of as a system of complex, interactive energy converters. Chemical energy is converted to mechanical power in muscle, sunlight is converted to chemical energy in photosynthesis, and an uncounted number of chemical to chemical transformations take place in active transport, oxidative phosphorylation, photosynthesis, and coupled enzyme processes. All of these processes are highly effective methods for converting energy.

Nonequilibrium thermodynamics has become a valuable tool in the study of coupled flow phenomena. With this approach, specific relationships are written between the flows and forces of a process. Simple expressions can then be derived for limiting operating states, the rates of energy flow needed to support these states, and various measures of the process effectiveness. The theory has been applied to many coupled biological phenomena.

Many biological energy converters are composed of large numbers of individual subunits. This is necessary because the capacity of a single subunit is usually not sufficient to maintain the levels of force or flow needed by the cell or organism. Groups of subunits also provide an additional regulatory mechanism in that individual subunits can be kinetically (phenomenologically) modified by covalent or noncovalent interactions, or by changes in the fractions of subunit isoenzymes.
The purpose of this research is to describe the property variations that occur because of changes in the types and numbers of the subunits that make up a system. Energy conversion in skeletal muscle is used as an example, but applications to other areas appear likely.
LITERATURE REVIEW AND BACKGROUND MATERIAL

Linear Nonequilibrium Thermodynamics

Basic concepts

Methods of analysis based on nonequilibrium thermodynamics are useful in describing processes that are dissipative in nature and which occur away from equilibrium. As living organisms are inherently of this character, nonequilibrium thermodynamic methods have been used to model many biological processes. Several books are available that describe the general field (38,100) as well as the more specific applications to biology (25,59). There are also chapters dedicated to the subject in many bioenergetic and physiology texts (81,122) and a few related review articles (24,126).

As an introduction to the method, a system is defined as a physical region of space in which some processes are occurring. An environment is also defined as a region surrounding the system that is large enough to contain all of the effects due to the system's processes. The system plus the environment are then equivalently isolated (see Figure 1), and the second law of thermodynamics can be written

\[ \frac{dS_{\text{tot}}}{dt} = \frac{dS_{\text{sys}}}{dt} + \frac{dS_{\text{envir}}}{dt} \geq 0 \]

where \( S \) represents the entropy, and the superscripts sys, envir, and tot refer to the system, environment, and system plus environment, respectively. The equal sign applies only in the limit of reversible processes.
FIGURE 1. The system and environment

It is customary to divide the system's entropy contribution into two terms, or

\[ dS^{sys} = d_{e}S^{sys} + d_{i}S^{sys} \]

where \( d_{e}S \) represents an entropy exchange with the environment and \( d_{i}S \) represents the entropy generated due to irreversible processes. If only the irreversible processes of the system are considered then

\[ dS^{envir} = -d_{e}S^{sys} \]

and the total entropy change due to the system's irreversibilities becomes

\[ dS^{tot} = dS^{sys} + dS^{envir} = d_{i}S^{sys} \]
In addition, if the processes of the system proceed at a steady rate (called a stationary state), then all of the system's time derivatives must be zero. Hence,
\[
\frac{dS_{sys}}{dt} = \frac{dV_{sys}}{dt} = \frac{dU_{sys}}{dt} = 0
\]
where \( V \) and \( U \) stand for the volume and internal energy, respectively.

The time derivative of the general Gibbs equation can be written for the environment as
\[
\frac{dU_{envir}}{dt} = T \frac{dS_{envir}}{dt} - P \frac{dV_{envir}}{dt} + \sum_{k} X_{k} \frac{dS_{envir}}{dt}
\]
In this expression, \( T \) stands for the temperature, \( P \) for the pressure, and each of the \( X_{k}(dS_{envir}^{k}/dt) \) terms represents a work term that is performed on the environment by the system. \( X_{k} \) is an environmental force and \( dS_{envir}^{k}/dt \) is the time derivative of the force's conjugated flow. Because of the isolation of the system plus surroundings, the time derivatives of the total energy and volume must be zero. For steady processes, since the system's derivatives must also be zero, it follows that
\[
\frac{dU_{envir}}{dt} = \frac{dV_{envir}}{dt} = 0
\]
Considering only the events of the environment that occur because of the system, the environmental rate of entropy increase must be equal to the total rate of entropy increase, or
\[
\frac{dS_{envir}}{dt} = \frac{dS_{tot}}{dt} = \frac{dS_{sys}}{dt}
\]
Substituting these relationships into the Gibbs equation gives
\[
\frac{dS^{\text{sys}}}{dt} = - \sum_{k} J_k \frac{\dot{\varepsilon}_k}{\dot{\varepsilon}_k} = \sum_{k} J_k X_k
\]

where

\[J_k = - \frac{\dot{\varepsilon}_k}{\dot{\varepsilon}_k}\]

This function is referred to as the dissipation function ($\Phi$), or

\[\Phi = \frac{dS^{\text{sys}}}{dt} = \sum_{k} J_k X_k\]

where each of the $J_k X_k$ terms represents a conjugated force-flow pair occurring at the system boundary. Using matrix notation for convenience, the dissipation function can be written

\[\Phi = J^T X\]

Here the underbars represent vectors or kx1 matrices, and the superscript $T$ stands for the transpose of a matrix. It should also be noted that the dissipation function can be derived on a microscopic scale (or material body) as opposed to the bulk derivation given here (59,81).

The basic premise of this method of analysis is that when thermodynamic forces are small (i.e., the processes are close to equilibrium), each of the flows can be described as a linear function of the forces on the system. For a system with $k$ force-flow pairs this can be expressed as

\[
\begin{align*}
J_1 &= L_{11} X_1 + L_{12} X_2 + \ldots + L_{1k} X_k \\
J_2 &= L_{21} X_1 + L_{22} X_2 + \ldots + L_{2k} X_k \\
&\vdots \\
J_k &= L_{k1} X_1 + L_{k2} X_2 + \ldots + L_{kk} X_k
\end{align*}
\]
or in matrix notation

\[ \mathbf{J} = \mathbf{L} \mathbf{X} \]

where \( \mathbf{L} \) is a \( k \times k \) square matrix. It is often convenient to express the forces as functions of the flows, and the equations can be written alternatively as

\[ \mathbf{X} = \mathbf{R} \mathbf{J} \]

where \( \mathbf{R} \) is the matrix inverse of \( \mathbf{L} \). These constitutive relationships are referred to as phenomenological equations, and the corresponding conductance or resistance coefficients are called phenomenological coefficients. If processes are occurring far away from equilibrium, where linear relationships do not describe the flows of forces, higher order terms are required often making these problems unwieldy.

Several single term phenomenological relationships are well-known. Ohm's law for the flow of current and Newton's law of cooling are examples on the macroscopic level, while Fick's law for diffusion and Fourier's law of heat transfer are microscopic examples. The classical engineering example of a coupled, two force-flow process is the Soret or Dufour effect that describes the coupling between mass and heat transfer (see Bird et al. (14)). Ultrafiltration, electro-osmosis, and centrifugation are other examples of coupled force-flow processes that have been analyzed using nonequilibrium thermodynamics (see Katchalsky and Curran (59)).

Thermodynamics places certain restrictions on the values of the phenomenological coefficients. First, by application of the statistical theory of fluctuations and microscopic reversibility,
Onsager showed that the coefficient matrix was symmetric, or

\[ L = L^T \]

This greatly reduces the number of coefficients needed to describe a coupled process. Next, by substituting the flow phenomenological equations, the dissipation function becomes

\[ \phi = X^T L X \geq 0 \]

For a \( k \) force-flow pair system, \( k \) phenomenological equations can be written; therefore, there are \( k \) degrees of freedom. If all of the forces are zero except the \( i \)th, the dissipation function becomes

\[ \phi = L_{ii} (X_i)^2 \geq 0 \]

and it can be concluded that \( L_{ii} \) is positive. In general then, all of the diagonal phenomenological elements must be positive since they are independent of the flows or forces. It can also be shown that the \( L \) matrix must be positive semi-definite, again a consequence of the dissipation function being nonnegative. Hence, every principal minor must also be nonnegative, or

\[ L_{ij} L_{ji} - L_{ii} L_{jj} \geq 0 \quad (i, j = 1, 2, \ldots, k) \]

which becomes using Onsager symmetry

\[ (L_{ij})^2 \geq L_{ii} L_{jj} \quad (i, j = 1, 2, \ldots, k) \]

Finally, in isotropic systems, there are some constraints on the types of coupling possible. These arise because of differences in the vectorial order of the force-flow pairs and are referred to collectively as the Curie-Prigogine principle \( (59, 85) \). While the forces and flows of many biological processes (muscular contraction, active transport, etc.) are of different vectorial order, the
anisotropy of these systems eliminates the restrictions inferred by this principle.

**Energy conversion**

**Definition** While the second law of thermodynamics states that the dissipation function must be positive, it does not exclude the possibility that some of the force-flow terms can be negative. In other words, some dissipative flows may cause other flows to move against their corresponding forces. When this occurs, the process is referred to as energy conversion.

For a two force-flow pair linear situation (see Figure 2), energy conversion occurs when one dissipation flow drives the other against its conjugate force. The dissipation function can be written

\[ \phi = J_1 X_1 + J_2 X_2 \]

with the corresponding phenomenological equations

\[ J_1 = L_{11} X_1 + L_{12} X_2 \]
\[ J_2 = L_{21} X_1 + L_{22} X_2 \]

Here the subscripts 1 and 2 refer to the output and input force-flow pairs, respectively. With this nomenclature, the criteria for energy conversion becomes

\[ J_1 X_1 \leq 0 \]
\[ J_2 X_2 > -J_1 X_1 > 0 \]

**Flow ratio versus force ratio** It is useful to consider the flow ratio and how this ratio depends on the force ratio. Dividing the output flow phenomenological equation by the input flow equation gives
FIGURE 2. A two force-flow pair energy converter

\[
\frac{J_1}{J_2} = \frac{\sqrt{L_{11}} \frac{x_1}{x_2} + \frac{L_{12}}{\sqrt{L_{11}L_{22}}}}{\sqrt{L_{11}L_{22}} \frac{x_1}{x_2} + \sqrt{\frac{L_{22}}{L_{11}}}}
\]

which describes how the flow ratio depends on the force ratio. By letting \( j \) and \( x \) stand for the flow and force ratio, respectively, and defining the two parameters

\[
q = \frac{L_{12}}{\sqrt{L_{11}L_{22}}}
\]

and

\[
z = \sqrt{\frac{L_{11}}{L_{22}}}
\]

this equation can be rewritten as

\[
j = \frac{zx + q}{qx + \frac{1}{z}}
\]
where Onsager symmetry \( (L_{12} = L_{21}) \) has been evoked. Dividing by \( Z \) nondimensionalizes the equation and gives

\[
\frac{1}{Z} = \frac{Zx + q}{qZx + 1}
\]

which can be used to plot the effect of coupling and the force ratio on the flow ratio as is shown in Figure 3.

The parameter \( q \), called the coupling coefficient or degree of coupling, was first defined by Kedem and Caplan (60). It is a measure of the relative magnitude of the nonconjugated to conjugated force's effect on the flows. From the phenomenological restrictions given earlier it is apparent that

\[-1 \leq q \leq 1\]

This parameter is an interesting measure of energy conversion. If \( q \) is equal to zero (no coupling, \( L_{12} = 0 \)) then the flow ratio becomes linearly dependent on the force ratio or

\[
j(q = 0) = Z^2x
\]

and each flow becomes independent of the presence of the nonconjugated force. No energy conversion can occur. At the other extreme as \( q \) approaches one, the flow ratio becomes

\[
j(q = 1) = Z
\]

and becomes constant or independent of the force ratio (because of this limit, \( Z \) has been referred to as describing 'phenomenological stoichiometry' (115)). A coupling coefficient of one does not necessarily imply that the processes are nondissipative. Complete coupling results in the phenomenological equations becoming linearly dependent (\( \text{det } L = 0 \)) and in the dissipation function contracting to a
FIGURE 3. Flow ratio versus force ratio
single but nonzero term. A nondissipative process will occur only in the reversible limit of infinitely small flows.

Using the criteria for energy conversion given above and the phenomenological equations, equivalent criteria can be derived in terms of the force or flow ratios, or

\[-1 \leq \frac{z_2}{q} \leq 0\]

\[0 \leq \frac{j}{2q} \leq 1\]

These restrictions indicate that energy conversion is restricted to the upper left quadrant of the flow ratio-force ratio diagram for positively coupled systems and to the lower right quadrant for negatively coupled systems. These regions are called driving regions, and the other regions of the diagram correspond to states where both force-flow pairs are dissipative.

**Limiting operating states**

There are two special sets of limiting operating states within the driving region. For a fixed input force, \(X_2\), when the force opposing the output flow is just large enough to eliminate that flow, the stationary state is referred to as a 'static head'. It should be noted that for incompletely coupled systems, energy must be expended to maintain a static head, even with no flow occurring. If the output force is reduced (or less negative), flow will occur, and if the force becomes zero, the flow will obtain its maximum in the energy conversion regime. This state is referred to as 'level flow'. Examples of static head states include fuel cells operating at open circuit, isometric muscle contractions, and membrane
systems maintaining constant concentration gradients, while short
circuit fuel cells, unloaded muscle contractions, and isotonic membrane
transport are all examples of level flow. These two sets of states are
represented by the two axis of the flow ratio diagram as is shown in
Figure 3.

**Other operating states** When energy conversion occurs away from
the limiting states described above, there is both an output force and
flow, and it is often convenient to describe these intermediate states
on a plot of the output flow against the output force. This type of
plot is called an output diagram. An engineering example of an output
diagram would be a plot of the pressure head developed versus the
volumetric flow rate for a centrifugal pump (see Section 6 (28)), while
the velocity-force diagram of muscle would be a biological example
(46).

For linear energy converters, the output flow is described by

$$J_1 = L_{11}X_1 + L_{12}X_2$$

If the driving force is held constant, it is apparent that energy
converters must operate in a linear fashion across the output diagram.
The nonlinearity expressed by a centrifugal pump is due to the
dependence of the phenomenological coefficients on the output force
(i.e., nonlinear behavior). Muscle also behaves in a nonlinear
fashion, but the deviation has been shown to be consistent with the
ideas of energy conversion systems to be presented later. Examples of
output diagrams are plotted in Figure 4.
Effectiveness of energy conversion

In discussing energy conversion it is often useful to have some description of the effectiveness of the conversion process. For systems operating at steady state, a converter efficiency can be defined as the power output \((-J_1X_1\)) divided by the power input \((J_2X_2)\), or

\[
\eta = -\frac{J_1X_1}{J_2X_2}
\]

Values of the efficiency can range from zero to one when the converter is operating in the driving region. Using the phenomenological equations, the flows can be eliminated to give

\[
\eta = -\frac{q + Zx}{q + \frac{1}{Zx}}
\]
which has been plotted in Figure 5 for positively coupled converters. Obviously, if either $J_1$ or $X_1$ is zero, then the efficiency must also be zero, and since it must have positive values between these limits, there must be a maximum. This maximum can be shown to be a function of only the degree of coupling and is given by

$$\eta_{\text{max}} = \frac{q^2}{(1 + \sqrt{1 - q^2})^2}$$

The locus of these maximum points can be expressed in terms of the force ratio as

$$\eta_{\text{max}} = (Zx)^2$$

which is also plotted in Figure 5.

It is important to point out that the efficiency depends not only on the phenomenological character of the energy converter but also on the operating state of the system (e.g., on the force ratio as derived above). Therefore, small values of the efficiency may not reflect the converter's ability to utilize a power source, but can also indicate that the converter is operating near a static head or level flow state. In fact, there are many in vivo biological systems that operate at very small or zero efficiencies (either $J_1 = 0$ or $X_1 = 0$).

For systems operating near static head, the force efficacy can be used as an effectiveness factor (43). This efficacy is defined as the force developed per unit of input power or

$$\varepsilon_{X_1} = \frac{X_1}{J_1 X_2}$$
FIGURE 5. Efficiency versus force ratio
For a constant input force, the force efficacy increases with increased output force and reaches its maximum at static head. Therefore, for systems where a large output force is desired, it is most effective to operate at static head.

A flow efficacy can also be defined as

\[ \varepsilon_{J_1} = \frac{J_1}{J_2 X_2} \]

for cases where large flows occur (43). It is a measure of the amount of flow that occurs per unit of input power. As with the force efficacy, this effectiveness factor reaches its maximum for level flow operating states.

Efficacies are useful effectiveness factors for systems operating at large flows or forces. Yet, the efficacies, like the efficiency, depend on the operating state of the system, and because they are dimensional, they cannot be used as a universal measure to compare different types of energy converters.

The coupling coefficient has proven to be a useful indicator of a process' ability to convert energy. Unlike the efficiency, the degree of coupling depends only on the phenomenological character of the system and not on the flows or forces. Like the efficiency, it is a dimensionless number whose value varies between zero and one, and as can be seen from Figure 5, increased coupling corresponds to increased efficiency at any given value of the force ratio. Also, as was shown above, the maximum level of efficiency and the degree of coupling are
related parameters. These properties make the coupling coefficient useful in describing the economy of a process.

Biological Energy Converters

There are many examples of energy conversion in biology. Muscle converts chemical energy into mechanical power. Chemical species are sequestered against their chemical potentials by either a chemical reaction (active transport) or the diffusion of some other species down its chemical potential. Both oxidative phosphorylation and photosynthesis generate proton gradients that are subsequently converted into useful chemical energy. In addition, numerous enzyme-catalyzed reactions are driven against their chemical affinities by coupling to a dissipative reaction.

In order to achieve the quantity of some desired force or flow, all of the systems described above are made up of large numbers of individual protein subunits. Often these subunits are oriented in a specific manner to facilitate the conversion process (active transport proteins are oriented within a membrane, muscle proteins are organized into filaments, etc.). In many biological energy conversion processes, the number of operating subunits can be varied as a control mechanism.

In addition to regulation by control of the number of subunits, biological systems are extensively regulated in other ways. The reversible binding of some regulatory chemical species can modify the kinetic (phenomenological) character of a protein. Hormone and divalent ion binding sites are examples of proteins regulated in this
manner. Many covalent modifications also occur such as phosphorylations and adenylations. It is also not unusual to find that the protein subunits are not all kinetically identical and that the cell can control the fraction of subunits, called isoenzymes, that make up a system. In some situations, more than one mechanism can be involved in a control scheme.

Enzymes that catalyze the first committed step of some metabolic sequence are often extensively regulated. As an example, consider the conversion of glutamate and ammonia into glutamine by the enzyme glutamine synthetase. This reaction is driven by the hydrolysis of adenosine triphosphate (ATP). Since glutamine is the major donor of nitrogen atoms in many biosynthetic pathways, its synthesis is feedback regulated by many nitrogen-containing products. Tryptophan, histidine, carbamoyl phosphate, glucosamine 6-phosphate, cytidine triphosphate (CTP), and adenosine monophosphate (AMP) are all known to regulate the catalytic activity of glutamine synthetase. In the biological synthesis of these compounds, at least one nitrogen atom is donated directly by glutamine. In addition, there are also inhibitor sites for the amino acids serine and glycine, each of which may indirectly receive its nitrogen atom from glutamine.

Systems can also be regulated by covalent modification. The glutamine synthetase enzyme described above can be modified by the attachment of an AMP unit to a specific tyrosine residue. The enzyme that catalyzes this reaction, adenylyl transferase, is also regulated by the addition of a uridylyl residue. Without the uridylyl group the
enzyme covalently attaches an AMP unit to glutamine synthetase, and in the modified state, it removes it. The effect of the added nucleoside is to enhance the depression of the previously described feedback inhibitors. The control mechanisms of glutamine synthetase is summarized in Figure 6.

Some systems may be composed of more than one type of energy conversion subunit that do the same job but differ kinetically. Muscle, for example, is known to contain different types of fibers with each fiber type containing different fractions of isoenzymes. Hence, muscles with different fiber compositions have different contractile properties.

In principle, a pair of two-flow energy conversion subunits can be connected in four (2^2) different ways. For each flow, the total flow can be either the sum of all of the converters (parallel flow) or can be the same as each of the converters (parallel flow). As is shown in Figure 7, the four different configurations possible are parallel-input parallel-output, parallel-input series-output, series-input parallel-output, and series-input series-output. If the subunits have more than two coupled flows, more configurations are possible (2^m configurations for m flow subunits).

More complex assemblages are also possible with more subunits (for a system of p subunits each with m different flows, p^m different configurations are possible). Yet, in most biological systems, groups of subunits tend to be connected in one of the four basic configurations described above. Complex systems are then constructed
FIGURE 6. The glutamine synthetase enzyme
FIGURE 7. Two-subunit two-flow energy conversion systems
from subsystems, which are themselves assembled in a parallel or series manner.

Intracellular active transport of a single species is an example of parallel-input parallel-output assembled energy converters that act as a system. An individual filament pair of muscle is orientated in a parallel-input series-output manner, but fibers and whole muscles are constructed by adding filament pairs in a complex fashion. This enables muscle to develop both the required output flow (velocity) and output force (tension).

Most biological processes are assembled in parallel-input fashion with the input flow being a chemical reaction or the dissipation of a chemical gradient. As a consequence, this work has concentrated on systems connected in a parallel-input manner. Output flows can be of a series nature, such as the velocity of cross-bridges in skeletal muscle, or of a parallel nature, as in the species flows in active transport. Since there is some evidence for subunit linearity in muscle, this system will be considered in more detail as an example of a parallel-input series-output energy conversion system.

Skeletal Muscle

Chapters on the biology of skeletal muscle can be found in various biochemistry and physiology texts (72,114) as well as in general articles (86). There are also more specific bioenergetic texts that review the thermodynamic aspects of contraction (25,122).
Skeletal muscle structure

Muscle tissue is made up of several bundles of muscle fibers called fasciculi. Each bundle contains many individual fibers that are formed by the fusion of myoblasts during the embryonic development of the organism. All of the fibers and bundles are held together by connective tissue: the endomysium surrounding individual fibers, the perimysium surrounding fasciculi, and the epimysium surrounding the entire muscle.

A muscle fiber contains many organelles. Each fiber has a plasma membrane called the sarcolemma and contains several myofibrils. These myofibrils are highly organized protein structures and are the contracting apparatus of the muscle fiber. Surrounding the individual myofibrils is an intracellular membrane sac called the sarcoplasmic reticulum. This membrane system is in close contact with the T-system, which is a series of invaginations of the sarcolemma that run perpendicularly through the muscle. The structure formed by the juxtaposition of these two membranes is referred to as a triad. Finally, muscle fibers also contain many nuclei, mitochondria, glycogen particles, and under certain conditions, Golgi apparatus and lysosomes.

When viewed with a light microscope, the myofibrils appear as a series of alternating light and dark bands called the I and A bands, respectively. Upon closer inspection, it can be seen that the I band is bisected by a narrow dark region called the Z-disk or Z-line, and the A band is bisected by a lighter region called the H-zone. The H-zone is itself bisected by a small, dark region called the M-line.
The fundamental contraction unit of muscle, called a sarcomere, is defined as that part of the myofibril that runs between adjacent Z-disks. Early structural studies have shown that each sarcomere contains a thin and thick set of overlapping protein filaments (51). In mammalian skeletal muscle, the bipolar thick filaments, which correspond to the A-band, are between 14 and 15 nanometers in diameter and about 1.55 micrometers long, while the thin filaments are 6 to 7 nanometers in diameter and about 1.0 micrometers in length. These thinner filaments are attached at one end to the Z-disk and run through half of the I band before becoming imbedded within the array of thick filaments. The portion of the A band that is void of thin filaments corresponds to the H-zone. An overall view of skeletal muscle is given in Figure 8.

The thin filaments from skeletal muscle are composed from four proteins: actin, tropomyosin, the troponin complex, and beta-actinin. At physiological conditions actin monomers polymerize to form F-actin filaments. These filaments, which make up the thin filament backbones, appear to be made up of two strands of actin monomers wound loosely around each other or, alternatively, can be viewed as a single strand of tightly wound subunits. This helical structure is not rigid and appears to have a random repeat length of between 35 and 38 nanometers (41).

Tropomyosin and the troponin complex are bound to the actin filament backbone. Tropomyosin has a two-chained alpha helical structure with a molecular weight of 68,000, a length of 42.3
FIGURE 8. Skeletal muscle
nanometers, and a diameter of 1.5 nanometers, and is bound to actin along the deep grooves that are formed by the actin monomers. One tropomyosin molecule runs along 13 to 14 actin monomer units and is associated with one troponin complex. This complex contains three proteins: troponin-I, troponin-C, and troponin-T. Troponin-C has a molecular weight of 18,000 and contains four calcium binding sites (two of which are important in the activation process). The others two components appear to be associated with anchoring troponin-C to actin and tropomyosin.

The nonfixed ends of the thin filaments appear to be capped with a protein that may inhibit the depolymerization or elongation of monomer units at the free thin filament ends, or may prevent the interaction of different thin filaments in severely shortened myofibrils (73). This protein, a dimer with subunit molecular weights of 37,000 and 34,000, is called beta-actinin.

The thick filaments of mammalian skeletal muscle are composed primarily of the proteins myosin and C-protein. Myosin is a large, asymmetric molecule that binds to F-actin filaments and has an ATPase that is actin activated. It has a molecular weight of 480,000 and is composed of two heavy chains of 200,000 daltons each and four light chains of approximately 20,000 daltons each. The two heavy chains form a long (150-160 nanometer), alpha-helical tail which separates on one end to form two globular heads. The location of the light chains is not precisely known, but a large body of evidence suggests that they are associated with the globular heads, possibly in the neck region,
where the alpha-helical tail separates. In electron micrographs, the centers of thick filaments appear relatively smooth while the midsection and ends are coarser. These rough regions correspond to the globular portions of the individual myosin molecules, which are packed into filaments so that the globular portions are separated by 14.3 nanometer intervals. Currently, there are two competing theories describing the organization of individual myosin molecules within the thick filaments (92,111).

Several distinct types of light chains have been isolated depending on the origin of the myosin. In fast-twitch skeletal muscle, there are three classes of light chains with apparent molecular weights of 25,000, 18,500, and 16,000. Each globular myosin head has one of the 18,500 dalton chains as half of its complement. Since there are two globular heads on every myosin molecule, three combinations of light chain structure are possible. Recent work suggests that all three are present in intact muscle (37,47,109). Slow-twitch skeletal muscle appears to have two different classes of light chains with molecular weights of 27,000 and one light chain class with a molecular weight of 19,000. Smooth muscle and nonmuscle myosins have two classes of light chains with apparent molecular weights of 20,000 and 17,000.

One class of light chain from each type of muscle has very similar properties. The molecular weight of these chains ranges from 18,500 to 20,000, all are able to bind Ca$^{2+}$, and all can substitute for the EDTA-removed light chain from scallop myosin. Also, all of these light chains can be phosphorylated by myosin light-chain kinases. This class
of light chains is commonly referred to as the regulatory, LC2, or DTNB light chains.

An additional 140,000 dalton protein, called C-protein, has been isolated from thick filaments. This protein appears to form seven bands around the outside of each half of the bipolar myosin thick filaments (32). Its function is unknown although several have been postulated (78,88).

Muscular contraction

Huxley proposed a sliding filament theory of muscle contraction that is now widely accepted (52). In this model, the myosin globular heads of the thick filaments interact with the neighboring thin filaments to form cross-bridges. These protein bridges, by undergoing a conformational change, pull the thin filaments toward the center of the sarcomere. The cross-bridges then break and the globular heads reset to prepare for another cycle.

ATP is now known to be indispensable for the activation process. Myosin hydrolyzes ATP rapidly, but the products of the hydrolysis, ADP and P\text{\text{\textsubscript{i}}}, remain tightly bound. These hydrolysis products are bound to the myosin heads when muscle is in the inactive state. During contraction, the myosin heads bind to specific locations along the thin filaments. This binding causes the ADP and P\text{\text{\textsubscript{i}}} products to be released, and the actomyosin complex undergoes a conformational change pulling the thin filament in toward the center of the sarcomere. This enables ATP to bind to myosin forcing the actomyosin complex to fall apart. The bound ATP is then hydrolyzed and the cycle repeats. This cycle is
discussed in many references (72,86,114) and is illustrated in Figure 9.

Early experiments by Ebashi have shown that Ca\(^{2+}\) plays an important role in the activation of contraction. At rest, Ca\(^{2+}\) is sequestered in the sarcoplasmic reticulum. When a nerve impulse reaches the muscle, the depolarization travels along the sarcolemma and down along the T-system. This depolarization causes the sarcoplasmic reticulum to release Ca\(^{2+}\). Although various theories have been presented (58,76), the Ca\(^{2+}\) release mechanism is still unknown. Recent work has shown that there are protein structures, possibly bridges, lining the sarcoplasmic reticulum where it is in close contact with the T-system (42,44). These proteins structures seem likely candidates to be involved in the process, but as to how they induce the Ca\(^{2+}\) release is still unclear.

Elevated levels of Ca\(^{2+}\) have been found to activate the contraction mechanism by a variety of methods depending on the type of muscle. Contraction is primarily controlled by the thin filament in vertebrate striated muscle. Two theories have been presented describing vertebrate smooth muscle activation: one involves myosin and the other actin. In invertebrate muscle, the control of contraction is either associated with myosin or with both myosin and actin.

A number of proteins are involved in the various activation schemes. Myosin activation mechanisms involve the LC2 light chains and fall into two classes: reversible Ca\(^{2+}\) binding or phosphorylation. A review of myosin controlled activation has recently been published
FIGURE 9. The actomyosin cross-bridge cycle
Actin controlled activation is usually associated with tropomyosin and the troponin complex, but smooth muscle activation may involve other proteins (see below).

In invertebrate molluscan muscle, it appears that Ca\textsuperscript{2+} released by the sarcoplasmic reticulum reversibly binds to the LC2 light chains. This binding appears to directly enable contraction (62).

A large body of evidence indicates that the activation of vertebrate smooth muscle is also myosin linked but is due to the phosphorylation of the regulatory light chains (2,27,53,108,110). Increased concentrations of sarcoplasmic Ca\textsuperscript{2+} appear to lead to the formation of a Ca\textsuperscript{2+}-protein complex. This Ca\textsuperscript{2+}-binding protein has been identified as calmodulin, a 17,500 dalton protein that is known to be involved in many Ca\textsuperscript{2+}-regulated systems. Two recent reviews have been published on this protein (123,132). The Ca\textsuperscript{2+}-calmodulin complex then binds to an enzyme called myosin light-chain kinase. This binding activates the kinase enabling it to phosphorylate the LC2 light chains allowing contraction to proceed. When the Ca\textsuperscript{2+} levels return to lower levels, phosphatase activity dephosphorylates the light chains and contraction stops.

It is interesting to note that myosin light-chain kinase from smooth muscle is phosphorylated by cyclic AMP-dependent protein kinase (1). This phosphorylation apparently blocks the binding of the Ca\textsuperscript{2+}-calmodulin complex to the light-chain kinase and may provide the mechanistic reason for the observed decrease in smooth muscle activity with increased cAMP levels.
The other postulated mechanism of smooth muscle control was presented by Ebashi and co-workers. In Ebashi's mechanism, a protein complex called leitonin is bound to the thin filaments in place of the troponin-complex (see Mikawa et al. (74,75)). \( \text{Ca}^{2+} \) reversibly binds to one of the two leitonin subunits releasing the inhibition to contraction.

Vertebrate striated muscle is activated by \( \text{Ca}^{2+} \) binding directly to the troponin-C of the thin filaments. When the free \( \text{Ca}^{2+} \) concentration is low, tropomyosin prevents the myosin heads from interacting with actin. The binding of \( \text{Ca}^{2+} \) to the troponin complex releases this inhibition letting the cross-bridges form. As in all of the other systems, \( \text{Ca}^{2+} \) is resequestered by an active transport system of the sarcoplasmic reticulum. This transport system lowers the free \( \text{Ca}^{2+} \) concentration when nerve stimulation ceases, and contraction stops. This mechanism is reviewed in many general articles and published lectures (40,72,114).

**Skeletal muscle regulatory processes**

Recent work indicates that there are several factors present in skeletal muscle that may act to regulate contraction properties. Skeletal myosin has retained both the \( \text{Ca}^{2+} \) binding sites and the mechanism of phosphorylation associated with activation of lower evolutionary muscle types. It is also known that there are several myosin isoenzymes which can change in response to the contraction environment of the fiber.
Skeletal muscle phosphorylation. It is now apparent that a light chain phosphorylation system, similar to the one in smooth muscle, exists in skeletal muscle. Perrie and co-workers first reported the existence of a phosphorylated form of the LC2 light chain in 1973 (93). This led to the isolation of a Ca^{2+}-dependent kinase (95), now called myosin light chain kinase, which was found to act specifically on myosin light chains (94). It was shown that this kinase was dependent on a Ca^{2+} binding factor (135), which was subsequently identified as calmodulin (12,87,134), the Ca^{2+}-binding protein that is also involved in smooth muscle contraction. Unlike smooth muscle light-chain kinase, this enzyme was not affected by cAMP or cAMP-dependent protein kinase (94). A phosphatase has also been isolated that is specific for the dephosphorylation of light chains (79). This mechanism is depicted in Figure 10.

Stimulation has been shown to be sufficient to cause light chain phosphorylation in rabbit (117,127), frog (7), rat (11,64,70), mouse (11,33), chicken (9), and human (50) muscle but not in fish muscle (133). In general, phosphorylation has been found to occur in skeletal fast twitch muscles but does not occur in slow twitch muscle. There has been one report of elevated levels of phosphorylation in the slow soleus muscle of the rabbit (127), but a recent study on the same muscle has not been able to duplicate this result (77).

Many investigations have been carried out to determine if phosphorylation has a physiological effect on skeletal muscle. A few studies have reported that phosphorylation has no effect on the ATPase
FIGURE 10. Mechanism of light chain phosphorylation
of purified myosin or heavy meromyosin (a proteolytic fragment of myosin that contains the globular heads) either in the absence or presence of actin filaments (79,116). Yet, Pemrick has reported otherwise (90) and has recently shown that the results are highly dependent on the experimental conditions (91).

In the rat extensor digitorum longus (EDL) muscle, the increased levels of phosphorylation have been correlated with post-tetanic potentiation of the twitch tension (64,71). This result has also been found in the human vastus lateralis (50) and rabbit plantaris (77) muscles. As with light chain phosphorylation, potentiation of twitch tension is found in fast-twitch muscle and not in slow-twitch muscle strongly suggesting that the two effects are related. One study has concluded otherwise reporting that, since the fall in potentiation and phosphorylation are not linearly related, the increased tension is not a result of phosphorylation (127). Yet, this conclusion may not be correct since the increase in potentiation has also been shown not to follow phosphorylation in a linear fashion (70). Nonlinear contraction property variations will be shown to occur naturally in the theoretical treatment to follow.

While conflicting evidence exists (11), it has been suggested that LC2 phosphorylation may correlate with an energy reduction in intact mouse muscle. Crow and Kushmerick (33) have reported that the energy use of the mouse EDL muscle decreases with prolonged stimulation (33). This reduced energy use plus a reduction in the unloaded contraction velocity was later found to be correlated with LC2 phosphorylation
Recent work by Barsotti and Butler (11) and Butler and co-workers (19) has shown that the situation may be more complex in that phosphorylation and the mechanical and energetic decreases appear to decouple when the muscle is contracted, allowed to rest, and contracted again to measure the muscle properties.

A similar phenomenon has been observed in the rabbit psoas muscle (31) in that there is a pronounced reduction in the energy cost of maintaining tension when the light chains are thiophosphorylated. Somewhat in contrast, another group has found that there are no mechanical changes in the same rabbit muscle with increased levels of phosphorylation (118). These results will be discussed later, but at this point it is sufficient to note that the differences are not phenomenologically in conflict.

Finally, although little quantitative data is available, the frog sartorius muscle is also known to undergo stimulation-dependent phosphorylation (7). In an earlier, unrelated study Kushmerick and Paul (67) have noticed a rapid and significant change in the rate of energy use of this muscle during sustained isometric contractions. It is interesting to speculate that light chain phosphorylation is also affecting the contraction properties of this muscle.

**Myosin reversible calcium binding** It is now widely accepted that skeletal myosin has two high affinity divalent ion binding sites (5,18,80) and that these sites are associated with the two LC2 light chains (3,80,125). In resting muscle, these sites would be predominantly occupied by Mg\(^2+\) ions, but when activated, the Mg\(^2+\) ions
would be partially displaced by Ca\(^{2+}\) (6). Morimoto and Harrington (80) have shown that Ca\(^{2+}\) binding modifies the hydrodynamic properties of myosin filament solutions, and others have shown that Ca\(^{2+}\) binding affects the proteolytic degradation of myosin by proteases (4). Properties of isolated light chains have also been shown to be affected by Ca\(^{2+}\) binding (3).

Although earlier studies have shown the opposite (18), Ca\(^{2+}\) binding appears to increase the pure actin-activated myosin ATPase at physiological ionic strength (29, 68, 101) suggesting that myosin might be dually activated. Yet, Bagshaw and Reed (6) have shown that the kinetics of Mg\(^{2+}\) dissociation are far too slow for Ca\(^{2+}\) binding to be an activating factor. Consequently, the current speculation is that Ca\(^{2+}\) binding may play a secondary or modulatory role.

It has been reported that the Ca\(^{2+}\) level in muscle influences the force-velocity relationship. While Ca\(^{2+}\) does effect the level of activation and, hence, all parameters that depend on the number of active cross-bridges, the activation level should not affect properties that are independent of the number of active cross-bridges (e.g., the maximum velocity of contraction). Any effect seen should be due to mechanisms unrelated to the activation process. Experiments performed by Julian (55, 56) indicate that the maximum velocity of contraction markedly decreases with decreased levels of Ca\(^{2+}\). Later, Moss (82) obtained similar results and proposed that Ca\(^{2+}\) binding to myosin or phosphorylation may be the underlying mechanism. On the other hand, Podolsky and Teichholz (98) and Thames et al. (120) have found no
effect in similar sets of experiments. To date, the differences have been unresolved, but Podolin and Ford (96) note that neither group has determined the levels of phosphorylation in their muscle samples, which may lead to the discrepancies. It should also be noted that the contraction behavior of partially active muscle may be different than that of fully activated muscle. If this occurs, then it will be difficult to determine the separate effects of activation and modulation.

Recent reports are still conflicting with two groups suggesting that $Ca^{2+}$ has no other effect than the simple on-off regulation the thin filaments (45,97). One group has suggested otherwise (83).

Since light chains may also be phosphorylated, efforts have begun to try to determine whether there is a combined effect of phosphorylation and ion binding. One study (49) indicates that phosphorylation has no effect on the $Ca^{2+}$ binding properties of rabbit skeletal myosin, while another indicates a slight decrease in the association constants (57) of both myosin and the individual light chains. A more substantial decrease has been reported elsewhere for isolated light chains (3). It has also been suggested that phosphorylation may be a necessary prerequisite for expression of actomyosin $Ca^{2+}$ sensitivity (101) although other explanations for this group's results are plausible. As will be implied later, continued research is still needed to determining whether there is a combined effect.
Skeletal muscle fiber and myosin isoenzymes

Not all of the fibers in skeletal muscle are identical. Indeed, few whole muscles are homogeneous in a single type of fiber. As a consequence, fibers have been classified by a number of criteria (histochemical staining, ultrastructure, etc.) which, in general, indicate the presence of three basic fiber types (see review by Close (30)). Barnard and co-workers (10) have referred to the three basic fiber types as fast-twitch white, fast-twitch red, and slow-twitch intermediate based on the histochemical staining for NADH-diaphorase and myosin ATPase. In this classification scheme, the histochemical properties of the two types of fast-twitch fibers differ, but many of the physiological properties do not. Because of this, it has become common to refer to fast-twitch red or fast-twitch white fibers simply as fast-twitch fibers, and slow-intermediate fibers as simply slow-twitch fibers. Fast-twitch fibers have faster speeds of shortening, higher Hill a/P0 force-velocity constants (see 46), and higher ATPase activities than slow-twitch fibers. In general, most muscles contain both types of fibers.

Since fiber types have different contraction properties, it should be expected that there would be differences in their respective contraction proteins. As mentioned above, fast-twitch myosin exists primarily in three isoenzyme forms (37,47)—the differences being attributed to the distribution of light chains (37,47,109). Slow-twitch myosin has been reported to exist in either one form (37) or two forms (47,89). Additionally, the electrophoretic mobilities of the myosin heavy chains from fast and slow fibers are different indicating
that myosins differ in more than light chain content (37). Recent evidence indicates that even individual fibers, while being classified as either fast- or slow-twitch, may contain myosins that are characteristic of both fiber types (69,103).

The pattern of nervous stimulation appears to determine the predominant isoenzyme of a fiber as indicated by stimulation pattern (107), denervation (26,105), denervation followed by reinnervation (26), disuse (112,121), and cross-reinnervation (8,124) studies. Additionally, there is a large body of evidence that indicates that different myosin isoenzymes are present during different developmental stages (36,48,104,113,128,130).

While many studies have pointed to the variability of myosin and to the physiological changes due to these variations, few studies have attempted to quantitatively correlate these changes. Moss and co-workers have shown that partial removal of the LC2 light chains reduced the maximum velocity of shortening of individual muscle fibers (84). This same group has shown more recently that the unloaded shortening velocity in fast muscle is correlated with the myosin heavy chain isoenzyme composition but not with the light chain content (103). The lack of correlation with the light chain content has been reported by others (54), and preliminary work by yet another group has indicated similar results (119). A similar phenomenon has been reported in developing slow twitch chicken muscle where the velocity of unloaded shortening has been related to the ratio of two types of myosin heavy chains (102).
Muscle Nonequilibrium Thermodynamics

The first application of nonequilibrium thermodynamics to muscular contraction was presented by Caplan in 1966 (21). In this model, Caplan considered whole muscle to behave as a system with a linear energy converter in series with a nonlinear regulator (see Figure 11). The dissipative function for the converter is written as

$$ \phi = V(-P) + vA $$

where $P$ is the muscle tension, $V$ is the contraction velocity, $A$ is the affinity of the ATP reaction, and $v$ is the reaction velocity. The phenomenological equations become

$$ V = L_{11}(-P) + L_{12}A $$

$$ v = L_{21}(-P) + L_{22}A $$

In Caplan's model, the regulator is fed information from the converter, which adjusts the converter input to the output load. Considering stability and requiring the response to be unique, Caplan was able to show that when the regulator was programmed with the simplest function (unity) that the system would operate to give an output equation of the form:

$$(P + a)(V + b) = (P_0 + a)b = (V_0 + b)a$$

where $P_0$ is the isometric tension ($V = 0$), $V_0$ is the unloaded contraction velocity ($P = 0$), and $a$ and $b$ are mechanical constants. This equation is identical to the Hill force-velocity equation (46), which is experimentally well-verified.

Later work by Wilkie and Woledge (129) showed that the relationship between the maximum efficiency and maximum power output
FIGURE 11. The Caplan muscle model

did appear to agree with the model. Unfortunately, the theory did not predict a relationship between the reaction velocity and the muscle load that was found experimentally from measurements of heat and work generation in muscle. It also predicted large variations in the chemical affinity of the ATP driving reaction that appeared unlikely.

Caplan defended his model (23) by stating that heat and work measurements may not be directly related to the reaction velocity and that the variation in reaction affinity occurs locally and is needed for muscle regulation. Heat and work measurements may be affected by the presence of other contraction related processes--for example, the uptake of $Ca^{2+}$ by the $Ca^{2+}$ pump of the sarcoplasmic reticulum, but it is unlikely that the rate of energy usage by this pump is dependent on the muscle tension as Caplan implies. Ostensibly, the variation in the reaction affinity occurs because of dissipation in the regulator. This cost of regulation appeared extremely high and unexpected considering that biological systems tend to be energy conserving. This has been an unsettling aspect of Caplan's model.

Bornhorst and Minardi (15) showed that integrated rate data could be predicted closely from nonequilibrium thermodynamics. Additionally,
the ratio of the reaction velocity between an unloaded isotonic and isometric contraction was reasonably close to the value suggested by Caplan (22).

These results led Bornhorst and Minardi to propose an alternative nonequilibrium thermodynamic model (16,17). This model differs from the original Caplan model in that the individual cross-bridges are each, on average, considered to act as linear energy converters. For each active cross-bridge, a dissipative function can be written as
\[ \phi^i = v^i\epsilon^i - P^i + v^i A^i \]
and the corresponding phenomenological equations are
\[ v^i = L^i_{21}(-P^i) + L^i_{22}A^i \]
\[ v^i = L^i_{11}(-P^i) + L^i_{12}A^i \]
where the superscript \( i \) refers to an individual cross-bridge property. A cross-bridge in this model is defined as being active if it is in the process of utilizing the input power source. This does not necessarily require that the cross-bridge be attached to the thin filament to be in the active state.

Because of the organization of the muscle filaments, each subunit force-flow property can be related to the corresponding property for the whole muscle. For example, the total tension and reaction velocity must be the sum of the individual cross-bridge contributions for each cross-bridge along a filament pair, or
\[ P = \sum_i p^i = nP^i \]
and
where \( n \) is the total number of active cross-bridges. In addition, since each cross-bridge is immersed in the same cytoplasmic fluid, the affinity of the driving reaction must be the same, and since each cross-bridge must be moving at the same velocity as that of the filament pair, the velocity of all of the active cross-bridges must be equal giving

\[
A = A^i
\]

and

\[
V = V^i
\]

respectively.

Assuming all of the cross-bridges are phenomenologically identical and using the restrictions given above to eliminate the individual cross-bridge properties gives

\[
\phi = \sum_i \phi^i = n \phi^i = V(-P) + vA
\]

for the total muscle dissipation function and

\[
V = L_{11} \frac{(-P)}{n} + L_{12} A
\]

\[
V = L_{21} (-P) + L_{22} nA
\]

for the 'overall' phenomenological equations. Here the phenomenological coefficients refer to the individual cross-bridge coefficients.

With this model, the nonlinear output behavior of muscle is accounted for by variations in the number of active cross-bridges,
which is a function of the muscle length and load. The dependence of the number of cross-bridges on the load is derived by assuming the force-velocity relationship of Hill (46) and the length variations are accounted for by considering the filament overlap (see 17). No explicit regulator is postulated.

Bornhorst and Minardi used these phenomenological expressions and the Hill equation to derive relationships for the heat and heat plus work rates as a function of the muscle load. These equations were found to agree with experimental data. An overall degree of coupling was also defined and was shown to be quite high, indicating that the conversion process is very effective. Later, chemical data were also found to agree with the theory (99).

This model has several conceptual advantages over the earlier one. First, the theory is grounded on the molecular cross-bridge theory of Huxley as opposed to the black box model of Caplan. Second, no abstract regulator was postulated, and hence, there is no dissipative cost of regulation. Finally, no widely varying reaction affinities are needed that are difficult to conceptualize as occurring within a cell.
PARALLEL AND SERIES ENERGY CONVERSION SYSTEMS

Simple Systems

Subunit dissipation function and phenomenological equations

All of the systems considered in this section consist of a fixed number of independently operating two-flow subunits driven by a constant input force. A subunit is defined as being a segment of some system that can preform energy conversion in the absence of the rest of the system. Each subunit is assumed to be phenomenologically unaffected by the presence of the other subunits, although the presence of additional subunits will effect the forces and flows associated with the individual converters. As was shown by Kedem and Katchalsky (61), it is possible to assemble systems where a subunit's presence can affect the phenomenological character of the other subunits. Systems of this type are not considered here.

Each subunit is assumed to behave in a linear fashion with one input force-flow term (subscripted 2) and one output force-flow term (subscripted 1). Consequently, the dissipation function and phenomenological equations are written as

\[ \phi^i = J^i_{11}X^i_1 + J^i_{22}X^i_2 \]

and

\[ J^i_1 = L^i_{11}X^i_1 + L^i_{12}X^i_2 \]

\[ J^i_2 = L^i_{21}X^i_1 + L^i_{22}X^i_2 \]

where the lower case index \(i\) represents a subunit property. It is
important to note that it is not possible at any time for the subunit dissipation functions to be negative since the subunits operate independently.

In many cases of biological interest, energy conversion is driven either by a chemical reaction or by the dissipation of some chemical gradient. Because both cases are examples of parallel-input behavior, the total input flow is the sum of the individual input flows. Correspondingly, the input force is the same for each of the subunits and is equal to the input force of the system. Mathematically

$$J_2 = \sum_i J_{2i}$$

and

$$X_2 = X_{2i} \quad \text{(for all } i)$$

where $J_2$ stands for the input flow, $X_2$ for the input force, and the lack of a superscript indicates a system property.

Since all of the systems considered in this section are input parallel, assemblies will be referred to only by the description of the output flow (e.g., a parallel-input series-output system will be referred to as a series-output system). In the section on more complex systems, the more complete nomenclature will be used.

For both the parallel and series situations, we wish to consider how a property of a system changes as a function of the types and numbers of subunits within that system. Phenomenological equations are derived first for systems of identical subunits and then for systems composed of two different types of subunits. Limiting operating
states, the input flows supporting these states, and coupling are then considered for the systems with two types of subunits. The corresponding equations for systems of identical subunits are easily derived from these.

**Parallel-output systems**

For a parallel-output system, the total output flow and force are given by

\[ J^i_1 = \sum_j J^i_j \]

and

\[ X^i_1 = X^i_1 \quad \text{(for all } j) \]

With the above force-flow restrictions and the subunit phenomenological relationships, a set of equations can be derived that relate the overall flows and forces. These equations are

\[ J_1 = n(L^i_{11}X_1^i + L^i_{12}X_2^i) \]

\[ J_2 = n(L^i_{21}X_1^i + L^i_{22}X_2^i) \]

where \( n \) is the number of subunits, all of which are assumed to be described by the same set of phenomenological equations. The overall dissipation function becomes

\[ \phi = \sum_i \phi^i = J^i_1X_1^i + J^i_2X^i_2 \]

Equations that relate overall flows to forces will be referred to as 'system' or 'overall' phenomenological equations.
It is apparent from these equations that when the number of operating subunits is not varying, the overall system is linear and is functionally identical to the operation of a single energy converter. The only difference between the overall system and an individual subunit is that each term is multiplied by the number of subunits. This enables this type of system to generate much greater output flows.

Our specific interest is in systems where not all of the subunits operate in a phenomenologically identical manner. The next more complex case would be a system that is composed of two distinct types of subunits (superscripted i and j). Two different sets of phenomenological equations and dissipation functions can be written—one set for each type of subunit. In this case, the overall flow equations become

\[ J_i = \sum_j J_i^j + \sum_i J_i^j = nx_i^i J_i^i + nx_i^j J_i^j \]

and

\[ J_j = \sum_i J_j^i + \sum_j J_j^j = nx_j^i J_j^i + nx_j^j J_j^j \]

where \(x_i^i\) and \(x_j^j\) represent the number fraction of \(i^{th}\) and \(j^{th}\) subunits and

\[ x_i^i + x_j^j = 1 \]

Both system forces are still equal to the forces of the individual subunits, or

\[ X_1 = X_1^i = X_1^j \quad \text{(for all } i \text{ and } j) \]

and

\[ X_2 = X_2^i = X_2^j \quad \text{(for all } i \text{ and } j) \]
Using these restrictions to again develop equations relating the system flows and forces gives

\[ J_1 = n(x^i_{11}x^i_{11} + x^i_{1i}x^j_{1i})X_1 + n(x^i_{12}x^i_{12} + x^i_{1j}x^j_{1j})X_2 \]

\[ J_2 = n(x^i_{21}x^i_{21} + x^i_{2i}x^j_{2i})X_1 + n(x^i_{22}x^i_{22} + x^i_{2j}x^j_{2j})X_2 \]

for the overall phenomenological equations. The total dissipation function is also given by

\[ \phi = \sum_i \phi_i^i + \sum_j \phi_j^j = J_1X_1 + J_2X_2 \]

These overall phenomenological equations exhibit several interesting properties. As a consequence of Onsager symmetry, it is apparent that the overall system is symmetric regardless of the number fraction of subunit types. Additionally, if the fraction of subunit types does not change over the period of experimental observation, then the equations will be effectively the same as the case of identical subunits. In this situation, the values of phenomenological coefficients derived from the effective model will be dependent upon the values of the subunit phenomenological coefficients and the fractional occurrence of each subunit type. It is also apparent that as the limit of a system composed of one type of subunit is approached, the equations reduce to the appropriate equations describing a system of identical subunits.

For this parallel-output assembly, the phenomenological terms are simply linearly weighted functions of the corresponding subunit types. And as in the case of identical subunits, each phenomenological term is multiplied by the number of subunits that compose the system.
Limiting operating states

As was discussed in the previous section, two limiting operating states exist for a two-flow energy converter. These states also exist for two-flow systems. A static head state for a system is defined as an operating state where no net flow is developed, while a level flow state refers to an operating state where no net force is produced.

Since our major concern is in describing the effect of changing the types of subunits within a system, it will be beneficial to normalize each property by that property's value for a system of one subunit type in order to study the effect of the second subunit type. This normalization has the advantage of combining the phenomenological coefficients into a reduced number of dimensionless groups, and it will also make special situations that arise because of special values of the coefficients more apparent. Four dimensionless coefficients are needed and are defined as

\[ a = \frac{L^{i}_{12}}{L^{j}_{12}} \]

\[ \beta = \frac{L^{i}_{11}}{L^{j}_{11}} \]

\[ q^{i} = \frac{L^{i}_{12}}{\sqrt{L^{i}_{11}L^{i}_{22}}} \]

and

\[ q^{j} = \frac{L^{j}_{12}}{\sqrt{L^{j}_{11}L^{j}_{22}}} \]
The last two coefficients are just the coupling coefficients of the individual converters, and the former two represent the ratio of the cross and output straight phenomenological terms. Both terms can be thought of as relative conductance terms describing the relative change in the output flow due to the corresponding force.

**Level flow** For the case at hand, the level flow state is described by

\[ J(X_1 = 0) = (x^i L_{12}^i + x^j L_{12}^j) nX_2 \]

It is apparent that the magnitude of this flow depends not only on the number of subunits and the magnitude of the input force but also on the fraction of subunit types that are present in the system. In normalized form this becomes

\[ \frac{J_1(X_1 = 0)}{J_1(X_1 = 0, x^i = 1)} = 1 + x^i(\alpha - 1) \]

The effect of changing the fraction of types of subunits in a parallel-output system can be seen to be linear with a slope that depends on the relative cross-conductance term. This relationship is plotted in Figure 12 for comparison with the series case.

An equation can also be derived which describes the input flow rate needed to maintain this operating state. Eliminating the first term in the second equation gives

\[ J_2(X_1 = 0) = (x^i L_{22}^i + x^j L_{22}^j) nX_2 \]

Since the input flows are parallel, this flow also depends on the number of operating subunits. After normalizing, this becomes
FIGURE 12. Parallel and series level flow
which shows that the input flow supporting level flow also varies in a linear fashion. For a system of identical subunits, flow equations for this operating state can be derived by letting either \( x^i \) or \( x^j \) be one in the above unnormalized equations.

**Static head**  
Static head occurs when the output force is just large enough to eliminate the output flow \( (J_1 = 0) \). Equations for this state are derived by setting the output flow equal to zero and solving for the output force. For the parallel case, this gives

\[
x_1(J_1 = 0) = \frac{(x^i L_{12} + x^j L_{12})}{(x^i L_{11} + x^j L_{11})} x_2
\]

In this situation, the force developed does not depend upon the number of subunits, and since no extreme points are possible, its magnitude is restricted between the values associated with the two types of subunits. This becomes clearer by inspecting the normalized equation which is given as

\[
x_1(J_1 = 0) = 1 + \frac{x^i (a - \beta)}{[1 + x^i (\beta - 1)]}
\]

Nonlinear variations are possible for this property in a parallel-output system and occur when the relative straight conductance term is not equal to one. In addition, a nonvariant static head occurs when the two conductance terms are equal. The possible responses have been plotted in Figure 13 for comparison with the series case.
FIGURE 13. Parallel and series static head
For this operating state, the assembly of the system requires that the output force be equal for each subunit even though the phenomenological character of some of the subunits may be different. As a consequence, the individual subunits do not have to be acting at their static head states, and individual flows can develop. These internal flows develop but such that the total flow from the system is zero. The resulting total force is produced to accommodate these conditions. Nonlinear changes in the static force occur because of unequal compensating changes in the individual flows relative to the force (i.e., $L_{11}^i = L_{11}^f$ or $\beta = 1$). One exception exists. If the cross and straight conductance coefficients are equal then the static force produced by the two types of subunits will be the same. The unequal straight output phenomenological coefficients are compensated by the variation in the cross coefficients. In this case, no variation in the static head occurs with changes in subunit type although other properties of the system will vary. No internal flows occur.

Because of the complex character of this operating state, it might be expected that the input flow supporting this state would also be complex. This flow is derived by eliminating the level of the static head between the two system phenomenological equations. After normalization, this becomes

$$J_2(J_1 = 0) = \frac{x^i \sigma^2}{\beta} \frac{l}{(q_j^i)^2} + \frac{(1 - x^i)}{(q_j^i)^2} \frac{(1 + x^i(\alpha - 1))^2}{[1 + x^i(\beta - 1)]}$$

$$J_2(J_1 = 0, x^i = 1) = \frac{1}{(q_j^i)^2 - 1}$$

It is obvious that this flow can also show nonlinear variations with
subunit type changes. Linear changes occur when the two relative conductance coefficients are equal, which corresponds to the case of no change in the static force. Since linear static force changes occur when $\beta$ is equal to one, it is quite possible to have nonlinear input variations giving rise to linear output changes.

**Other operating states** When operating between the static head and level flow states, energy conversion devices produce both a force and flow. For systems with a fixed input force, linear output curves occur only when the number of subunits and the fractions of individual subunit types remain constant. Hence, it is possible for phenomenologically linear systems to give nonlinear output curves by controlling the number or types of active subunits in the system.

It is apparent that the output flow increases with an increased numbers of subunits for parallel-output systems, while the force does not. The opposite will be found for series systems discussed below. Hence, systems can be built from subunits to cover virtually the entire output space by building composite parallel and series assemblies.

**System coupling** The degree of coupling was first developed by considering a description of the flow ratio as a function of the force ratio. For systems of energy converters, an overall coupling coefficient can be defined in a similar manner. It can be shown that, regardless of the assemblage of subunits, this overall parameter does not depend on the number of operating subunits but only on the individual phenomenological coefficients and the fractions of subunit types. In the simpler case of a system of identical subunits, the
overall coupling coefficient is identical to the coupling of the individual subunit.

Parallel assemblies of subunits have an overall coupling coefficient of the form

\[
Q = \frac{[1 + x^i(\alpha - 1)]}{\sqrt{[1 + x^i(\beta - 1)][\frac{x^2}{\beta} \frac{1}{(q_i^2)^2} + (1 - x^i) \frac{1}{(q_j^2)^2}]}}
\]

This function can exhibit a single extreme point in respect to changes in subunit fractions. It can also be proved that it is not possible for a maximum to occur across the physical domain of subunit fractions \((0 \leq x^i \leq 1, \text{for all } x^i)\). Hence, it is not possible for the system coupling to be greater than the coupling of the most effective subunit type within the system. The same applies for the maximum efficiency. The opposite is not true, and it is possible for the coupling to be less than that of the subunits (i.e., a minimum point is possible). The criterion for this occurring in a system of two subunit types is

\[
\frac{[\frac{\alpha}{\beta} - 1]^2}{[1 - (\frac{q^1}{q^c})^2]} > 1
\]

where \(q^1\) and \(q^c\) are the coupling coefficients of the least and most tightly coupled subunits and \(\alpha\) and \(\beta\) are defined as

\[
\alpha = \frac{L^1_{12}}{L^c_{12}}
\]

and
respectively. This ineffectiveness is another indication that phenomenologically distinct subunits tend to be counteractive in trying to maintain some fixed operating states.

**Series-output systems**

In series-output systems, it is not the output flow that is additive but the output force. The system output flows and forces are given by

\[ J_1 = J_i^1 \quad \text{(for all } i) \]

and

\[ X_1 = \sum_i X_i^1 \]

where the summation is over all of the subunits within the system. Taking the individual converters as operating linearly as before and assuming that they are all identical, the system phenomenological equations can be derived as

\[ J_1 = L_{11}^i \frac{X_1}{n} + L_{12}^i X_2 \]

\[ J_2 = L_{21}^i X_1 + L_{22}^i nX_2 \]

The system dissipation function becomes

\[ \Phi = \sum_i \Phi_i = J_1 X_1 + J_2 X_2 \]

which is the same as before. These equations are identical to those used by Bornhorst and Minardi (17) to describe muscle contraction.
If the system is composed of two different types of subunits \((i,j)\), the output force becomes

\[ X_1 = \sum_i X_i + \sum_j X_j = x^i nX^i_1 + x^j nX^j_1 \]

while the output flow remains constant. In this case, the overall phenomenological equations become

\[
J_1 = \left[ \frac{x^i}{L^i_{11}} + \frac{x^j}{L^j_{11}} \right]^{-1} \frac{X_1}{n} + \frac{\frac{x^i L^i_{12}}{L^i_{11}} + \frac{x^j L^j_{12}}{L^j_{11}}}{\left[ \frac{x^i}{L^i_{11}} + \frac{x^j}{L^j_{11}} \right]}
\]

\[
J_2 = \frac{x^i L^i_{12} + x^j L^j_{12}}{L^i_{11} + L^j_{11}} X_1 + \frac{x^i [L^i_{22} - \frac{L^i_{12} L^i_{21}}{L^i_{11}}]}{L^i_{11}} + \frac{x^j [L^j_{22} - \frac{L^j_{12} L^j_{21}}{L^j_{11}}]}{L^j_{11}} + \frac{x^i L^i_{12} + x^j L^j_{12}}{L^i_{11} + L^j_{11}} nX_2
\]

and the dissipation function is again just the sum of the overall force-flow products.

These equations appear considerably different from the parallel-output equations. In terms of the overall flows and forces, each flow is still linearly related to the forces, yet each term is not simply weighted by the number of subunits— a consequence of both flows not being assembled in an identical fashion. Individual phenomenological terms are more complex in that each is dependent on more than the
corresponding individual phenomenological coefficients unlike the case with parallel-output systems (e.g., the cross term is dependent on the individual straight input coefficients).

Limiting operating states

Level flow The level flow state occurs when the output force is zero, or

\[ J_1(X_1 = 0) = \frac{x^i}{L_{11}} \times \frac{x^j}{L_{12}} \times \frac{L_{11}^j}{L_{12}^i} X_2 \]

Using the same dimensionless coefficients, this can be expressed as

\[ J_1(X_1 = 0, x^i = 1) = 1 + \frac{x^i(\alpha - 1)}{[\beta + x^i(1 - \beta)]} \]

The maximum flow in this case again depends on the affinity but not on the number of operating subunits. While the flow can exhibit nonlinear behavior with subunit changes, no extremes are possible, and therefore its value must lie between the values for systems composed of all i and j type subunits. It is also possible for the value of the relative straight conductance term to be so large or small that essentially no change will occur with subunit changes over most of the possible type region. Systems can then be built which operate at essentially the same level flow, but differ in other respects.

This situation is similar to the static head operating state for a parallel-output system. In this case, it is the flow that is required to be identical for each of the subunits while the total force must be
zero. Since the phenomenological character of the subunits is not the same, the individual converters will adjust their force development such that the output flows are equal. This results in forces being developed internally but with a net force of zero. The nonlinear changes associated with this operating state are a consequence of the differences in the compensating abilities of the subunit types. Linear changes can only occur when the straight phenomenological coefficient are the same (i.e., $L_{11}^i = L_{11}^j$ or $\beta = 1$).

An equation for the input flow that maintains this state can be derived as

$$J_2(X_1 = 0) = \left[ x^i \left[ \frac{L_{12}^i - \frac{L_{12}^i L_{21}^i}{L_{11}^i}}{L_{11}^i} \right] + x^j \left[ \frac{L_{12}^j - \frac{L_{12}^j L_{21}^j}{L_{11}^j}}{L_{11}^j} \right] + \frac{x^i L_{12}^i L_{11}^i}{L_{11}^i} + \frac{x^j L_{12}^j L_{11}^j}{L_{11}^j} \right] n X_2 \left[ \frac{x^i}{L_{11}^i} + \frac{x^j}{L_{11}^j} \right]$$

which again depends on the number of operating subunits. In normalized form this becomes

$$\frac{J_2(X_1 = 0)}{J_2(X_1 = 0, x^i = 1)} = (q^2) \left[ \frac{x^i a^2}{\beta} \left[ \frac{1}{(q^i)^2} - 1 \right] + (1 - x^i) \left[ \frac{1}{(q^j)^2} - 1 \right] + \frac{1}{\beta} \left[ \beta + x^i (a - \beta) \right]^2 \left[ \beta + x^j (1 - \beta) \right] \right]$$

As was the case for the input flow associated with the parallel-output system static head, only when $a = \beta = 1$ will the profile be linear across the subunit regime. This corresponds to an output flow that
does not change with subunit changes. It is also possible to have linear output flow changes with the input flow varying nonlinearly ($\beta = 1, \alpha \neq 1$).

**Static head**

The static force developed can be expressed as

$$X_1(J_1 = 0) = -\left[ \frac{x^i L_{12}}{L_{11}} + \frac{x^j L_{12}}{L_{11}} \right] nX_2$$

which becomes

$$\frac{X_1(J_1 = 0)}{X_1(J_1 = 0, x^i = 1)} = 1 + x^i \left( \frac{\alpha}{\beta} - 1 \right)$$

after normalization. The magnitude of the static head depends on the number of subunits since the forces are additive in series systems. The change in static force developed with changes in subunit composition is linear and depends on the ratio of the two normalized cross-conductance terms. As in the parallel case, if the two coefficients are equal, even though different than one, the force does not change with subunit changes. In other words, if the ratio of the phenomenological coefficients of the two terms in the first equation is the same, the static head developed is unaffected by changes in the fractions of subunits.

The input flow that supports this force level is derived by combining the two phenomenological equations to eliminate the static force. Normalizing the resulting equation gives

$$\frac{J_2(J_1 = 0)}{J_2(J_1 = 0, x^i = 1)} = 1 + x^i \left[ \frac{\alpha^2}{\beta \left( \frac{1}{(q^i)^2} - 1 \right)} \left( \frac{1}{(q^i)^2} - 1 \right) - 1 \right]$$
As in the parallel case, when the output property can only vary in a linear fashion with subunit changes, the associated input flow also varies in a linear manner.

**System output diagrams** The output diagram for systems of this type will be linear only for nonchanging fractions and numbers of subunit types. In contrast with the parallel case, it is the force level that increases within increased numbers of subunits, while the flow is restricted to be of the order of magnitude of a subunit.

**System coupling** For systems assembled in a series-output manner, the system coupling is given by

\[
Q = \frac{[\beta + x^i(\alpha - \beta)]}{[\beta + x^i(1 - \beta)]} \sqrt{\frac{\beta}{[\beta + x^i(1 - \beta)]} \left[ \frac{x^i \alpha^2}{[\frac{q^i}{\beta}]^2} + \frac{(1 - x^i)}{[\frac{q^i}{\beta}]^2} - \frac{x^i(1 - x^i)(1 - \alpha)^2}{[\beta + x^i(1 - \beta)]} \right]}
\]

As for the parallel-output case, this measure of effectiveness cannot exhibit a maximum, but can exhibit a single minimum in the valid subunit fraction range (0 ≤ x^i ≤ 1 for all i). The criterion for this minimum occurring is

\[
(a - 1)^2 \left[ \frac{(q^i)^2 \left(1 - \left(\frac{1}{q^i}\right)^2\right)}{(q^i)^2 - \left(\frac{1}{q^i}\right)^2} \right] > 1
\]

where the same definitions carry over from the previous section.
More Complex Situations

The previous section describes only the relatively simple case of two types of subunits each with a single input and output flow. At least two more complex types of systems can exist.

First, it is possible for a system to contain more than two types of subunits. In the most general case, the parallel-input series-output phenomenological equations become

\[ J_1 = \left[ \sum_{i}^{m} \frac{x_i}{L_{11}} \right]^{-1} X_1 + \frac{m \times \frac{i}{L_{12}}}{\sum_{i}^{m} \frac{x_i}{L_{11}}} X_2 \]

\[ J_2 = \frac{m \times \frac{i}{L_{21}}}{\sum_{i}^{m} \frac{x_i}{L_{11}}} X_1 + \left[ \sum_{i}^{m} \frac{x_i}{L_{22}} - \frac{x_i}{L_{11}} \right] + \frac{m \times \frac{i}{L_{12}} \times \frac{i}{L_{12}}}{\sum_{i}^{m} \frac{x_i}{L_{11}}} \]

Here the summations are over the \( m \) different types of subunits that make up the system. Note that in all cases, if the subunit types, even though different, do not change over the experiments of interest, then these equations will behave like the single subunit type phenomenological equations. The descriptions of limiting operating states, the flows supporting these states, and system coupling are easily extended to these cases, and many of the properties described
above in the two type of subunit carry over into these more complex situations.

Considering a three subunit type parallel-input series-output system in more detail, the maximum output flow \(X_1 = 0\) is given by

\[
J_1(X_1 = 0) = \frac{x^i L_{12} + x^j L_{12} + x^k L_{12}}{L_{11} L_{11} L_{11}} X_2
\]

where \(i, j, \text{ and } k\) represent three types of subunits. The behavior of this system property can be described as a surface in a three-dimensional prism space. Each of the bounding surfaces of this space describes the behavior of the corresponding two subunit type system, and if a triangular topographical map is made, the lines corresponding to fixed values of the maximum output flow can be shown to be linear. Figure 14 illustrates this situation.

In some cases, it is common to find complex systems of this type where only a single subunit type is varied and the rest are not changed. When this occurs, the complete phenomenological equations can be reduced to an equivalent set that describe the relative changes in just the varying subunit type (see Figure 14). The resulting lumped coefficients are themselves dependent on the subunit types and the occurrence of the nonvarying types in the system. For the three subunit type example considered above, any vertical cut through the prism space represents this situation, and two classes of lumped subunit types result—each class being composed of a fixed fraction of
FIGURE 14. A three subunit type parallel-series system
two subunit types. This situation will be of interest in the next section.

Systems can also exist that have more than two coupled forces driving flows. As an example, consider a three flow parallel-parallel-parallel system where one dissipative flow drives the other flows against their corresponding forces. If this system is linear in the forces, the phenomenological equations become

\[
J_1 = n(L_{11}^1X_1 + L_{12}^1X_2 + L_{13}^1X_3) \\
J_2 = n(L_{12}^1X_2 + L_{22}^1X_2 + L_{23}^1X_3) \\
J_3 = n(L_{31}^1X_1 + L_{32}^1X_2 + L_{33}^1X_3)
\]

where the subscripts 1 and 2 indicate the output flow-force pairs, and the subscript 3 indicates the driving flow-force pair. In this equation all of the subunits are assumed phenomenologically identical. If the subunits are not all identical, then each of the above phenomenological terms would be a linearly weighted sum of the individual coefficients that make up the system. Coupling in multiple flow energy conversion has been discussed by Caplan (20).

A biological example of this situation is the well-studied sodium-potassium active transport pump. In this system, the hydrolysis of ATP drives the flow of sodium from the cell and the flow of potassium into the cell.
AN EXAMPLE - MUSCULAR CONTRACTION

Myosin Light Chain Phosphorylation

Mouse extensor digitorum longus (EDL) muscle

Kushmerick and Crow have reported that sharp decreases in the maximum velocity of contraction and the isometric rate of energy use occur in the mouse EDL muscle during long sustained contractions (33,35). They have also noted that during these physiological changes the LC2 light chains become progressively more phosphorylated, suggesting that phosphorylation may lead to changes in the character of some of the muscle cross-bridges (34,35).

As was discussed above, an actomyosin filament pair can be viewed as a parallel-input series-output energy conversion system with each cross-bridge being considered as a subunit. To extend the model to a whole muscle, the appropriate contributions due to the presence of each filament pair must be added. In effect, this is the assembly of a composite system (muscle) from subsystems (filament pairs). Assuming the subsystems in this assembly are identical, the whole muscle representation is similar to that for the filament pairs except for a normalization that is needed to account for muscle size variations.

This example cannot be considered identical to the two subunit type system described in the previous section for two reasons. First, all of the systems considered earlier operated with a constant number of active subunits. This is not the case in muscle, where from geometric considerations the number of cross-bridges would depend on
the degree of filament pair overlap as well as the muscle velocity or load. Since it is not possible to control, or even measure, the number of active cross-bridges during a contraction, application of these thermodynamic models is restricted to the muscle properties that are not dependent on the number of active cross-bridges. A second problem exists in that the maximum extent of phosphorylation in this muscle was found to be 52(±5) percent, including an indigenous phosphorylation level of 11(±4) percent. Because not all of the cross-bridges are phosphorylated during a contraction, any model must account for the presence of at least three subunit types. Fortunately, as was discussed above, this more complex model can be simplified for the case at hand.

Phenomenological equations The appropriate model for describing the phosphorylation effects would consist of one type of subunit that can exist in either of two stimulation-dependent states (unphosphorylated or phosphorylated) and one type of subunit that is not able to be phosphorylated during contraction. This latter cross-bridge type includes all of the cross-bridges that are not modified during stimulation, including any indigenously phosphorylated cross-bridges. Although this class of cross-bridges is heterogeneous, since the fractions within this group are not changing over the duration of the experiments, these cross-bridges can be lumped into a single class. Each of the constant phenomenological coefficients of this subunit class is then some function of the individual cross-bridge coefficients weighted to the extent to which they occur within the muscle.
The appropriate model for this situation consists of three types of subunits. The phenomenological equations become

\[ V = \left[ \frac{\frac{x^v}{L_{11}^v} + \frac{(1-z)x^u}{L_{11}^u} + \frac{zx^u}{L_{11}^{u,p}}} {n} \right] (-P) + \]

\[ \left[ \frac{x^v L_{12}^v} {L_{11}^v} + \frac{(1-z)x^u L_{12}^u} {L_{11}^u} + \frac{zx^u L_{12}^{u,p}} {L_{11}^{u,p}} \right] \]

\[ \left[ \frac{x^v}{L_{11}^v} + \frac{(1-z)x^u}{L_{11}^u} + \frac{zx^u}{L_{11}^{u,p}} \right] \]

\[ A \]

\[ V = \left[ \frac{x^v L_{21}^v} {L_{11}^v} + \frac{(1-z)x^u L_{21}^u} {L_{11}^u} + \frac{zx^u L_{21}^{u,p}} {L_{11}^{u,p}} \right] \]

\[ \left[ \frac{x^v}{L_{11}^v} + \frac{(1-z)x^u}{L_{11}^u} + \frac{zx^u}{L_{11}^{u,p}} \right] \]

\[ (-P) + \left[ x^v \left[ L_{22}^v - \frac{L_{12}^v L_{21}^v}{L_{11}^v} \right] \right. \]

\[ + (1-z)x^u \left[ L_{22}^u - \frac{L_{12}^u L_{21}^u}{L_{11}^u} \right] + zx^u \left[ L_{22}^{u,p} - \frac{L_{12}^{u,p} L_{21}^{u,p}}{L_{11}^{u,p}} \right] + \]

\[ \left[ \frac{x^v L_{12}^v} {L_{11}^v} + \frac{(1-z)x^u L_{12}^u} {L_{11}^u} + \frac{zx^u L_{12}^{u,p}} {L_{11}^{u,p}} \right] \]

\[ \left[ \frac{x^v L_{21}^v} {L_{11}^v} + \frac{(1-z)x^u L_{21}^u} {L_{11}^u} + \frac{zx^u L_{21}^{u,p}} {L_{11}^{u,p}} \right] \]

\[ \left[ \frac{x^v}{L_{11}^v} + \frac{(1-z)x^u}{L_{11}^u} + \frac{zx^u}{L_{11}^{u,p}} \right] \]

\[ nA \]

where \( v \) represents the stimulation-invariant cross-bridges, and \( u \) and \( u,p \) represent the stimulation-variant nonphosphorylated and phosphorylated cross-bridges, respectively. \( z \) represents the relative extent of phosphorylation.
In order to study the effect of phosphorylation, it would be desirable to combine many of the constant coefficients and invariant fractions into lumped constants—leaving explicit only that part of the model that is due to phosphorylation. In order to simplify this model, the following coefficients are defined

\[
L_{11}^U = \left( \frac{x_v}{L_{11}^V} + \frac{x_u}{L_{11}^U} \right)^{-1}
\]

\[
L_{11}^P = \left( \frac{x_v}{L_{11}^V} + \frac{x_u}{L_{11}^P} \right)^{-1}
\]

\[
L_{12}^U = \left[ \frac{x_v}{L_{11}^V} + \frac{x_u}{L_{11}^U} \right] \left( \frac{x_v^L}{L_{12}^V} + \frac{x_u^L}{L_{12}^U} \right)
\]

\[
L_{12}^P = \left[ \frac{x_v}{L_{11}^V} + \frac{x_u}{L_{11}^P} \right] \left( \frac{x_v^L}{L_{12}^V} + \frac{x_u^L}{L_{12}^P} \right)
\]

\[
L_{21}^U = \left[ \frac{x_v}{L_{11}^V} + \frac{x_u}{L_{11}^U} \right] \left( \frac{x_v^L}{L_{21}^V} + \frac{x_u^L}{L_{21}^U} \right)
\]

\[
L_{21}^P = \left[ \frac{x_v}{L_{11}^V} + \frac{x_u}{L_{11}^P} \right] \left( \frac{x_v^L}{L_{21}^V} + \frac{x_u^L}{L_{21}^P} \right)
\]
Each of these coefficients depends only on the phenomenological coefficients and the number fraction of stimulation-independent cross-bridges \((x^u = 1 - x^v)\). Hence, each of these coefficients is a constant. Introducing these lumped coefficients into the above phenomenological equations gives

\[
V = \left[ \frac{(1 - z)}{L_{11}^U} + \frac{z}{L_{11}^P} \right] \left[ \frac{L_{11}^U}{L_{11}^P} \right]^{-1} \left[ \frac{(1 - z)}{L_{11}^U} + \frac{z}{L_{11}^P} \right] A
\]
These equations are identical to the phenomenological relations for the two subunit model described before but with the variable $x^i$ or $x^j$ being replaced by $z$, and the $i$ and $j$ type coefficients being replaced by $U$ and $P$ coefficients. In reducing this model to this form, each of the simple coefficients takes on a specific character. For the $U$ type coefficients, this character is one of a mixture of the phenomenological character of the stimulation-independent and stimulation-dependent unphosphorylated cross-bridges. The $P$ coefficients have properties that are representative of the stimulation-independent and stimulation-dependent phosphorylated cross-bridges. Because each of the resulting coefficients has some character of the time-independent type of cross-bridges, the actual variation in phenomenological properties can be greater than the variation expressed by lumped coefficients. In terms of the prism space discussed in the previous section, this lumping represents a cut through the space at the plane where the fraction of stimulation-independent subunits is a constant.
Also from the previous section it is apparent that four coefficients need be determined in order to use the theory. A value of the phosphorylated coupling coefficient \( q^p \) is obtainable directly from the information provided by Kushmerick and Crow. The unphosphorylated coupling coefficient \( q^u \) can be calculated if a value for the maximum speed of shortening were known for the unphosphorylated (relative) muscle, or alternatively, it can be calculated from the cross-conductance term. This latter approach will be used. Both conductance terms will be obtained by regression of one of the muscle property variations that is not dependent on the number of active cross-bridges.

The most convenient way to obtain these coefficients is by regression of the maximum velocity of contraction data. This choice was based on dependence of the function on fewer parameters and the relatively uncomplicated nature of the function in respect to these parameters. Once these parameter estimates are obtained, the theory can be compared with the isometric rate of energy use data, overall coupling can be described, and the variation in the number of cross-bridges can be estimated.

**Maximum contraction velocity** The maximum contraction velocity occurs when the muscle tension is zero. Using the first phenomenological equation and setting the muscle tension to zero gives

\[
V_M = \frac{\frac{(1 - z)L_u^{12} + zL_p^{12}}{L_{11}^U}}{\frac{1 - z}{L_{11}^U} + \frac{z}{L_{11}^P}} \text{A}
\]

\[
V_M = \frac{(1 - z)L_u^{12} + zL_p^{12}}{(1 - z)L_{11}^U + zL_{11}^P} \text{A}
\]
where the subscript M indicates an unloaded isotonic contraction state. By normalizing this velocity to its value at complete phosphorylation, this becomes

$$\frac{V_M}{V_M(z = 1)} = 1 + \frac{(1 - z)(a - 1)}{\beta + (1 - z)(1 - \beta)}$$

where the previously defined $a$ and $\beta$ have been carried over. Hence,

$$\alpha = \frac{U}{L_{12}}$$

and

$$\beta = \frac{L_{11}}{L_P}$$

It is important to note that since the maximum velocity of contraction does not depend upon the number of active subunits, it would not be dependent upon the extent of overlap of the filaments.

The maximum contraction velocity data derived from Crow's data are given in Table 1 (personal communication, M. T. Crow, Division of Oncology, Stanford University School of Medical, February 1984).

Subtracting the indigenous level of phosphorylation and normalizing the extent of phosphorylation to its maximum yields the relative extent of phosphorylation or

$$z = \frac{x^{Pi} - x^{Pi(int)}}{x^{Pi(max)} - x^{Pi(int)}}$$

It is assumed that the fraction of active phosphorylated cross-bridges is equal to the total fraction of phosphorylated myosin heads.
TABLE 1. Contraction velocity versus phosphorylation data.

<table>
<thead>
<tr>
<th>Extent of Phosphorylation $^a$</th>
<th>Relative Extent of Phosphorylation $^b$</th>
<th>Maximum Velocity$^a$ (1/sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.118 (0.055)</td>
<td>0.00 (0.194)</td>
<td>----</td>
</tr>
<tr>
<td>0.130 (0.026)</td>
<td>0.030 (0.148)</td>
<td>6.38 (0.15)</td>
</tr>
<tr>
<td>0.271 (0.028)</td>
<td>0.381 (0.121)</td>
<td>5.74 (0.13)</td>
</tr>
<tr>
<td>0.420 (0.035)</td>
<td>0.751 (0.139)</td>
<td>5.05 (0.07)</td>
</tr>
<tr>
<td>0.506 (0.050)</td>
<td>0.965 (0.181)</td>
<td>4.09 (0.04)</td>
</tr>
<tr>
<td>0.514 (0.034)</td>
<td>0.985 (0.160)</td>
<td>3.19 (0.04)</td>
</tr>
<tr>
<td>0.520 (0.055)</td>
<td>1.000 (0.191)</td>
<td>3.21 (0.04)</td>
</tr>
</tbody>
</table>

$^a$Parentheses indicate standard deviations.

$^b$Parentheses indicate estimated standard deviations derived as outlined by Young (136).

The data were directly fitted with a nonlinear regression package (see 106) using two different schemes (Marquardt and Gauss-Newton). The regressions were weighted to the inverse of the variances since the usual assumption of equal variances did not appear to hold (see Table 1). Both methods gave identical results starting from several initial estimates of the parameters indicating that the methods were not regressing to a local minimum in the residual. A plot of this curve and the data are given in Figure 15 and the parameters developed are given in Table 2. It is apparent from this figure that the model is able to account for the contractile velocity variations.

Values for the cross phenomenological coefficients can be obtained when values for the maximum contraction velocities and the reaction
FIGURE 15. Maximum velocity versus relative phosphorylation
TABLE 2. Nondimensional phenomenological constants

<table>
<thead>
<tr>
<th>Regression method</th>
<th>Alpha^a</th>
<th>Beta^a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Marquardt</td>
<td>1.90 (0.13)</td>
<td>0.158 (0.077)</td>
</tr>
<tr>
<td>Gauss-Newton</td>
<td>1.90 (0.13)</td>
<td>0.158 (0.077)</td>
</tr>
</tbody>
</table>

^a Parentheses indicate standard errors.

affinity are known. A reasonable estimated value for the hydrolysis of ATP at cellular conditions would be 10.0 kcal/mole or \(4.18 \times 10^4\) \((N\cdot m)/mole\) (123,16). This gives

\[
L_{12}^U = \frac{V_M(z = 0)}{A} = 1.46 \times 10^{-4} \text{ moles/(N\cdot m\cdot sec)}
\]

and

\[
L_{12}^P = \frac{V_M(z = 1)}{A} = 7.66 \times 10^{-5} \text{ moles/(N\cdot m\cdot sec)}
\]

From this figure it is also clear that as phosphorylation occurs, the contraction speed drops and that the effect becomes more pronounced as phosphorylation increases. This reduction in the contraction speed is probably not a detriment to the organism since phosphorylation occurs during long sustained contractions as opposed to the short unloaded contractions that would be associated with quick movement. While some elevated level of phosphorylation is undoubtedly present during these types of contraction, it is apparent from Figure 15 that partial levels of phosphorylation effect the contraction speed only
slightly. Contraction speeds decline severely only when the phosphorylation approaches completion.

Isometric rate of energy use The rate of energy use equation derived in the last chapter is based on the number of active subunits, which cannot be measured in muscle. Rederiving this equation in terms of the isometric muscle tension gives

\[
\nu_o = \frac{z(L_{22} - \frac{(L_{12})^2}{L_{11}^P}) + (1 - z)(L_{22}^U - \frac{(L_{12}^U)^2}{L_{11}^U})}{zL_{12}^P + (1 - z)L_{12}^U - \frac{z}{P} \frac{L_{11}^P}{L_{11}^U}}
\]

where the subscript \( o \) indicates the isometric state. After normalization this becomes

\[
\frac{\nu_o}{P_o} = \frac{z\beta \left[ -\frac{1}{(q')^2} - 1 \right] + (1 - z)\alpha^2[\frac{1}{(q')^2} - 1]}{[z\beta + (1 - z)\alpha][\frac{1}{(q')^2} - 1]}
\]

If there were no phenomenological cross-bridge variations, the isometric reaction could be written in terms of the isometric tension as

\[
\nu_o = (\frac{L_{11}L_{22}}{L_{12}} - L_{12})P_o
\]

which becomes when integrated

\[
\Delta\nu_{Pi} = \int_0^t \nu_o \, dt = (\frac{L_{11}L_{22}}{L_{12}} - L_{12}) \int_0^t P_o \, dt
\]

where \( \Delta\nu_{Pi} \) denotes the total energy used by the muscle. Hence, a plot of the total energy use versus the tension-time integral would be
expected to be linear. For the case at hand, this equation would apply in both the limiting cases of no and complete phosphorylation. Since it appear that there are only small effects associated with low levels of phosphorylation (see Figures 15 and 18), this plot would be expected to be linear initially with a slope equal to $v_o/P_o (z = 0)$. As the phosphorylation completes, it would again be expected that the rate of energy use would become constant giving a linear relationship. This plot is provided by Crow and Kushmerick (33) for the mouse EDL muscle and, as can be seen in Figure 16, does approach such a constant slope as the tension-time integral increases. These authors have conveniently provided linear relations for these two approximately straight portions of the curve (see Table 3). Two different sets of correlations are shown because the original purpose of this work was to show that there was an energy balance between the chemical energy used and the oxygen recovery in this muscle. Using the average slopes, the two sets give

$$v_o/P_o (z = 0) = 23.2 \times 10^{-6} \text{ moles/(N\cdot m\cdot sec)}$$

and

$$v_o/P_o (z = 1) = 11.2 \times 10^{-6} \text{ moles/(N\cdot m\cdot sec)}$$

from which the individual coupling coefficients can be found to be

$q_u = 0.928$

and

$q_p = 0.934$
TABLE 3. Energy use-tension-time integral relations

<table>
<thead>
<tr>
<th></th>
<th>EDL</th>
<th>Soleus</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Oxygen Recovery Data</strong></td>
<td>$\Delta P_i = 0.71(1.4) + 23.4(3.55) /Pdt$</td>
<td>$\Delta P_i = 0.36(0.75) + 8.73(0.51) /Pdt$</td>
</tr>
<tr>
<td></td>
<td>$0 &lt; /Pdt &lt; 0.788$</td>
<td>$1.05 &lt; /Pdt &lt; 2.62$</td>
</tr>
<tr>
<td></td>
<td>$11.7(1.6) + 23.4(3.55) /Pdt$</td>
<td>$12.4(1.3) + 11.4(1.2) /Pdt$</td>
</tr>
<tr>
<td><strong>Chemical Change Data</strong></td>
<td>$\Delta P_i = 0.10(0.42) + 22.9(2.3) /Pdt$</td>
<td>$\Delta P_i = 0.03(0.36) + 8.75(0.98) /Pdt$</td>
</tr>
<tr>
<td></td>
<td>$0 &lt; /Pdt &lt; 0.788$</td>
<td>$0 &lt; /Pdt &lt; 2.62$</td>
</tr>
<tr>
<td></td>
<td>$12.4(1.3) + 11.4(1.2) /Pdt$</td>
<td>$12.1(1.0) + 11.2(0.9) /Pdt$</td>
</tr>
<tr>
<td><strong>Averaged Relations</strong></td>
<td>$\Delta P_i = 0.40(0.73) + 23.2(2.1) /Pdt$</td>
<td>$\Delta P_i = 0.20(0.42) + 8.74(0.55) /Pdt$</td>
</tr>
<tr>
<td></td>
<td>$0 &lt; /Pdt &lt; 0.788$</td>
<td>$1.05 &lt; /Pdt &lt; 2.62$</td>
</tr>
<tr>
<td></td>
<td>$12.1(1.0) + 11.2(0.9) /Pdt$</td>
<td>$12.1(1.0) + 11.2(0.9) /Pdt$</td>
</tr>
</tbody>
</table>

*Parentheses indicate standard errors.

b*Parentheses indicate standard errors estimated as described by Young (136).
FIGURE 16. Energy use versus tension-time integral
The theoretical curve for the normalized isometric reaction rate to tension ratio as a function of the relative extent of phosphorylation is plotted in Figure 17 using these parameters. Experimental energy rates were estimated by Kushmerick and Crow by averaging the total energy used over a three second time interval divided by the tension-time integral over the same time interval. The authors did not provide explicit tension versus time data but did state that the tension decreased only slowly and in a linear fashion over the period of interest. The tension-time integral can then be approximated as the tension at the center of the time interval multiplied by the time difference across the interval or

\[
\frac{\Delta \nu P_1 | t_2}{\int_{t_1}^{t_2} P_0 dt} = \frac{\Delta \nu P_1 | t_2}{P_0 \left| \frac{t_1 + t_2}{2} \right| (t_2 - t_1)} = \frac{\nu_0 | t_1 + t_2}{2}
\]

Therefore, the energy use rates provided by Kushmerick and Crow represent approximate differential rate data. The phosphorylation levels at the centers of the time intervals were approximated by averaging (interpolating) the phosphorylation levels of the ends of the intervals. Alternatively, it was found that the phosphorylation data correlated well with a one-parameter empirical model of the form

\[ z = 1 - (1 + kt)e^{-kt} \]

where \( k = 0.455 \) (see (39) and Figure 18). Either method produces essentially identical phosphorylation levels at the centers of the time intervals. A summary of these data is given in Table 4.
FIGURE 17. Time variation of phosphorylation
TABLE 4. Approximated rate of energy use data

<table>
<thead>
<tr>
<th>Time Interval Center (sec)</th>
<th>Relative Extent of Phosphorylation of Energy Use(^a) x 10(^4) (mole/(N·m·sec))</th>
<th>Normalized Rate of Energy Use(^b) x 10(^4) (mole/(N·m·sec))</th>
<th>Oxygen Recovery</th>
<th>Chemical Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-3</td>
<td>1.5</td>
<td>0.190 (.114)</td>
<td>2.15 (.40)</td>
<td>1.97 (.23)</td>
</tr>
<tr>
<td>3-6</td>
<td>4.5</td>
<td>0.566 (.092)</td>
<td>1.73 (.26)</td>
<td>1.71 (.22)</td>
</tr>
<tr>
<td>6-9</td>
<td>7.5</td>
<td>0.858 (.114)</td>
<td>1.32 (.38)</td>
<td>1.29 (.22)</td>
</tr>
<tr>
<td>9-12</td>
<td>10.5</td>
<td>0.975 (.121)</td>
<td>1.05 (.32)</td>
<td>0.938 (.15)</td>
</tr>
<tr>
<td>12-15</td>
<td>13.5</td>
<td>0.992 (.125)</td>
<td>0.893 (.14)</td>
<td>1.02 (.17)</td>
</tr>
</tbody>
</table>

\(^a\)Parentheses indicate standard deviations.

\(^b\)Parentheses indicate estimated standard deviations derived as outlined by Young (136).

From the plot it is apparent that at intermediate levels of phosphorylation, the experimental data points fall beneath the model. Most of the deviation is believed to be due to the characteristic smoothing that results when estimating rate data in a differential manner. This problem is expected to be especially prevalent since the estimates are doubly smoothed—once by considering the tension-time integral as an estimate of differential rate data and once by considering the differential rate data as an estimate of \(v_0/P_0\). In addition, the value of \(\beta\), due to the deviation of the contraction velocity data and the sensitivity of the model to this parameter, is subject to some degree of uncertainty (see the standard error given in...
FIGURE 18. Rate of energy use versus relative phosphorylation
Table 2). Since this constant can contribute to a small extent to the amount of curvature in the energy rate model, this may also be a small contributing factor. Yet even with the small discrepancies, the comparison is promising in that the accelerating decreasing trend was found.

As can be seen from the figure, phosphorylation results in a substantial reduction in the cost of maintaining tension. For a completely phosphorylated muscle, this can cut the cost of sustaining contraction by a considerable degree. It is apparent that the cost of attaching a phosphate group to the light chain is more than offset by the energy saving associated with the contraction changes. This appears to be the physiological purpose for skeletal muscle light chain phosphorylation.

It is also worth noting that if the cross-bridges are not changing in character over the time duration of a contraction, then the total energy use versus tension-time integral plot would be expected to remain linear. Data provided by Kushmerick and Crow for the soleus mouse muscle illustrates this linearity (see Figure 16 and Table 3). In this muscle, unlike the EDL muscle, no changes were found in either the level of phosphorylation or the chemical or mechanical contraction properties. This result is an additional piece of supporting evidence for the use of nonequilibrium thermodynamics in describing muscle contraction.

**Overall muscle coupling** The overall coupling coefficient for this series output system can be derived as
\[
Q = \left[ (1 - z)a + z\beta \right] \left[ (1 - z) + z\beta \right] \left[ z\beta \left( \frac{1}{(q')^2} - 1 \right) + (1 - z)a \right]^{1/2}
\]

Using the values given above for the nondimensional coefficients, the system coupling can be plotted as a function of the extent of phosphorylation as is shown in Figure 19. Although the limiting values of the individual coupling coefficients are lower than those reported for frog and tortoise muscle (16), the coupling of the EDL mouse muscle is still quite tight being greater than 0.93 with a corresponding maximum efficiency of 0.46. Given the variances associated with the experimental data, the individual lumped cross-bridge coupling coefficients are most likely not significantly different. This may be inherent, or the result of each effective cross-bridge type containing 60 percent of the character of the same type of cross-bridge. It is interesting that during the phosphorylation process the coupling coefficient expresses a minimum. But this probably has little physiological significance since partial levels of phosphorylation do not appear to be intended operating states but only transient states that the muscle goes through to reach the more economic state of complete phosphorylation.

The coupling coefficient and maximum efficiency for the mouse soleus muscle were also found to be quite high. Using the data of Kushmerick and Crow (from Table 3), the coupling coefficient was found to be 0.92, giving a maximum efficiency of 0.44.
FIGURE 19. Muscle coupling versus relative phosphorylation
A value of contraction efficiency can also be obtained by measuring the heat and work generated in an active muscle. This efficiency is not identical to the efficiency reported here, but differs by the ratio of the enthalpy to free energy ratio of the ATP reaction. Considering this factor, the values of efficiency obtained here from the nonequilibrium theory appear to be in close agreement with the values obtained by making heat and work measurements.

**Variation of cross-bridge numbers** Although the tension versus time data were not explicitly provided by Kushmerick and Crow, they did state that during the isometric contractions, the tension initially remained steady followed by a small steady decrease that never exceeded 20 percent of the maximum tension. This decrease was assumed to indicate the onset of fatigue, but the possibility that phosphorylation could also effect the isometric tension cannot be excluded. In any case, the two effects cannot be separated. Hence, the possibility of fatigue will be ignored.

Rearranging the first phenomenological equation for an isometric contraction \( V = 0 \) and normalizing to the state of complete relative phosphorylation level gives

\[
\frac{P_o/n_o}{P_o/n_o (z = 1)} = \frac{\alpha}{\beta} + z(1 - \frac{\alpha}{\beta})
\]

which becomes for the unphosphorylated muscle

\[
\frac{P_o/n_o (z = 0)}{P_o/n_o (z = 1)} = \frac{\alpha}{\beta}
\]

By neglecting fatigue, the tension changes by only 20 percent, or
\[
\frac{P_o(z = 0)}{P_o(z = 1)} \approx 1.25
\]

and substitution gives

\[
\frac{n_o(z = 1)}{n_o(z = 0)} \approx 10
\]

for the ratio of active cross-bridges. This implies that the number of cross-bridges operating during an isometric contraction with complete relative phosphorylation is ten times that operating when there is no relative phosphorylation. It is important to note that this does not mean that there are ten times the number of attached cross-bridges. At least a portion of the difference is probably due to a lengthening of the reset period after cross-bridge detachment (see Figure 9). In addition, the fatigue factor has been neglected, and the result is highly dependent on the regressed value of the straight conductance term. Yet it does suggest that there can be rather striking differences in the components and intermediates present in actively contracting muscle.

**Recent work** Recent work by Barsotti and Butler (11) and Butler and co-workers (19) suggests that phosphorylation may not be the sole contributor to the stimulation-dependent variations that occur in EDL mouse muscle. In certain experiments, these groups have been able to decouple the decreases in contractile properties and the phosphorylation phenomenon. Specifically, they found that at high levels of phosphorylation the maximum velocity of contraction remained high if, after an isometric contraction, the muscle was allowed to rest.
before the measurement of the muscle speed. The level of phosphorylation remains high because of the relatively slow dephosphorylation by light chain phosphatases. Similar results were found when the isometric rate of energy use was measured.

The implication is that the regulatory trigger may not be a simple phosphorylation-dephosphorylation but might be more complex. The most likely other candidate for being involved in the modulation is the well-documented effect of Ca\(^{2+}\) binding to the myosin LC2 light chains (see literature review). It is unlikely that Ca\(^{2+}\) alone acts as the modulator since the partial activation experiments suggest that Ca\(^{2+}\) binding either acts as an activator (not an inhibitor) (55,56,82,83) or has no effect (45,97,98,120). One postulate that has not been thoroughly studied is the possibility that both phosphorylation and Ca\(^{2+}\) binding may be necessary for the cross-bridges to be phenomenologically different.

The Ca\(^{2+}\) binding has been excluded as a contraction activating factor because of the relatively slow binding time (~1.0 seconds) in comparison to the activation time of skeletal muscle (~0.1 seconds). In resting muscle, where the free Ca\(^{2+}\) concentration is very low (10\(^{-7}\) M), most of the divalent binding sites are occupied by Mg\(^{2+}\), which has a much higher in vivo concentration (10\(^{-3}\) M). In active muscle because of the Ca\(^{2+}\) released by the sarcoplasmic reticulum, the free Ca\(^{2+}\) concentration is increased (1.5 x 10\(^{-5}\) M) and is sufficient to displace some of the bound Mg\(^{2+}\). Bennett and Bagshaw (13) have reported kinetic dissociation constants for both Ca\(^{2+}\) and Mg\(^{2+}\).
Assuming reversible binding of both ions, the relative change in cross-bridges that are Ca²⁺ bound can be derived as

\[ \frac{\text{Ca}^{2+}}{\text{Ca}^{2+} + \text{Ca}^{2+} (\text{int})} = 1 - e^{-Bt} \]

where

\[ B = \frac{[\text{Ca}^{2+}] (f_{\text{Mg}} - 1)}{K_{\text{Ca}} f_{\text{Mg}}} \]

In this derivation, it has been assumed that the addition of the exchanged Mg²⁺ ions does not significantly change the free Mg²⁺ concentration. This assumption is justified because of the larger free Mg²⁺ concentrations in the sarcoplasm in comparison to the concentration of myosin heads with Mg²⁺ bound. In these equations \( k_{\text{Ca}} \), \( K_{\text{Ca}} \) and \([\text{Ca}^{2+}] \) stand for the kinetic dissociation rate constant, the equilibrium dissociation constant, and the concentration of Ca²⁺ in active muscle. The modifying function, \( f_{\text{Mg}} \), depends on the Mg²⁺ binding properties and intracellular Mg²⁺ concentration. This function is

\[ f_{\text{Mg}} = \frac{K_{\text{Mg}}}{[\text{Mg}^{2+}]} + 1 \]

where \( K_{\text{Mg}} \) and \([\text{Mg}^{2+}] \) represent the equilibrium dissociation constant and the intracellular Mg²⁺ concentration. Reasonable values for the constants involved in these equations are given in Table 5. The Ca²⁺ response is plotted in Figure 20 along with the phosphorylation versus time correlation discussed earlier.
If the changes in the EDL muscle are due to the composite effect of phosphorylation and Ca\(^{2+}\) binding, then for the thermodynamic model the relative levels of P\(_i\)-Ca\(^{2+}\) cross-bridges can be given as

\[
\frac{z_{P_i-Ca^{2+}}}{x} = \frac{x_{Ca^{2+}}}{x_{Ca^{2+}}^{(max)}} - \frac{x_{P_i}}{x_{P_i}^{(int)}}
\]

where each process is assumed to occur independently. This function is also plotted in Figure 20. As can be seen from this figure, since the Ca\(^{2+}\) binding occurs much more rapidly than the phosphorylation, the relative extent of P\(_i\)-Ca\(^{2+}\) cross-bridges is essentially identical to the relative extent of phosphorylated cross-bridges. Consequently, the previous graphs and constants derived based only on the extent of phosphorylation will not have to be corrected to account for the Ca\(^{2+}\) binding.

The data presented by Barsotti and Butler (11) and Butler and coworkers (19) can now be explained. Long stimulation leads to high levels of phosphorylated and Ca\(^{2+}\) bound cross-bridges and to the observed changes noted by Kushmerick and Crow. Letting the muscle rest (8 seconds) results in low levels of Ca\(^{2+}\) binding but not in phosphorylation because the kinetic activity of the specific phosphatases is relatively slow. Then stimulating the muscle for only a short time (0.5 seconds) and measuring the contraction velocity and energy use results in high values because there is not a sufficient amount of Ca\(^{2+}\) bound. Using the thermodynamic model for the maximum contraction velocity along with the kinetic model for Ca\(^{2+}\) binding results in only a seven percent change in the maximal contraction
FIGURE 20. Time dependent stimulation induced LC2 changes
TABLE 5. Constants associated with myosin-Ca$^{2+}$ binding.

<table>
<thead>
<tr>
<th>Intracellular concentrations</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Resting [Ca$^{2+}$]</td>
<td>$1.0 \times 10^{-7}$ M</td>
</tr>
<tr>
<td>Active [Ca$^{2+}$]</td>
<td>$1.5 \times 10^{-5}$ M</td>
</tr>
<tr>
<td>[Mg$^{2+}$]</td>
<td>$1.0 \times 10^{-3}$ M</td>
</tr>
</tbody>
</table>

Equilibrium (dissociation) constants [49]

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>$K_{Ca}$</td>
<td>$3.3 \times 10^{-8}$ M</td>
</tr>
<tr>
<td>$K_{Mg}$</td>
<td>$4.0 \times 10^{-6}$ M</td>
</tr>
</tbody>
</table>

Kinetic dissociation constants [13]

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>$k_{Ca}$</td>
<td>0.70 sec$^{-1}$</td>
</tr>
<tr>
<td>$k_{Mg}$</td>
<td>0.051 sec$^{-1}$</td>
</tr>
</tbody>
</table>

velocity (see Figure 21). This estimate is conservative in two respects. First, phosphorylation is assumed to remain maximal after an eight second rest, and second, an actual step change in the Ca$^{2+}$ concentration has been assumed. Both approximations suggest that the actual contraction velocity change would be even smaller.

A set of experiments is suggested by this hypothesis. It would be of interest to see if the contraction speed would fall as predicted by the curve of Figure 21 when muscle samples were contracted to complete
phosphorylation, allowed to rest, and contracted again for various durations before measuring the contraction speed. It could be argued that the quicker contraction decreases were due to the increased effect of fatigue on the samples. A check on this would be to simply allow the muscle to rest for a second time then follow the fall in contraction velocity again. If an even faster decrease is noticed, then fatigue has become a factor. If the fall in velocity again follows Figure 21 then the results suggest that $\text{Ca}^{2+}$ binding is an important cross-bridge modifying process.

**Rabbit psoas muscle**

As was mentioned in the literature section, light chain phosphorylation has also been found in chemically skinned rabbit muscle. Cooke and co-workers (31) report that thiophosphorylation of the myosin light chains occurs in stimulated skinned rabbit psoas fibers (a thiophosphate derivative was used because it cannot be removed from the light chains by light chain phosphatase). This modification was found to be associated with a decrease in the energy cost of maintaining tension. The ATP cost decreased by a factor of two while the development of tension was unaffected. This decrease is similar to that reported by Crow and Kushmerick in the mouse EDL muscle (33,34).

In contrast, Sweeney and Kushmerick have recently reported stimulation dependent phosphorylation in skinned rabbit fibers (118). No effect on the maximum velocity of contraction was found.
NORMALIZED MAXIMUM
CONTRACTION VELOCITY

RELATIVE EXTENT OF LIGHT-
CHAIN MODIFICATION
In comparing these results, the two sets of data appear contradictory. This can be shown to be not necessarily true from the thermodynamic model. The maximum contraction velocity relation derived in the previous section in the limiting case of no phosphorylation is given by

\[ \frac{V_H(z = 0)}{V_H(z = 1)} = \alpha \]

From Sweeney and Kushmerick's data the relative cross-conductance term must be one (no change in the contraction speed). The model equation for the isometric rate of ATP hydrolysis in the same limiting state is

\[ \frac{v_o/P_o(z = 0)}{v_o/P_o(z = 1)} = \frac{\alpha \left[ \frac{1}{(v)2} - 1 \right]}{\left[ \frac{1}{(p)2} - 1 \right]} \]

Hence, it is apparent that changes in the isometric input flow rate can occur when the relative cross-conductance term is equal to one. This happens when the coupling coefficient of the two types of cross-bridges are different. For a nonphosphorylated cross-bridge coupling of 0.95, a phosphorylated cross-bridge coupling of 0.974, or a 2.5 percent change, would account for the difference.

It should also be pointed out that phosphorylated and thiophosphorylated cross-bridges have been assumed phenomenologically identical. It is difficult to draw any conclusions about the validity of this assumption since a comparison of the two types of modification has not been made. Consequently, the differences seen may be the result of the different chemical modification.
It is also interesting to speculate about the differences seen in the mouse and rabbit muscle's response to phosphorylation. The dissimilar experimental conditions must be considered an important contributing factor. By using whole muscle, the mouse EDL experiments were conducted nearer the physiological operating condition. The temperature of these experiments was 20°C. In the rabbit experiments chemically skinned fibers were used, which enables control of the bathing solution around the myofibrils but is unquestionably further removed from physiological conditions. The temperature of the rabbit experiments also had to be reduced to 4°C in order to stabilize the skinned fibers. In light of Pemrick's data on isolated myosin fragments (91), different results using skinned muscle fibers cannot be unexpected.

**Frog sartorius muscle**

Qualitatively, light chain phosphorylation has been exhibited in the frog sartorius muscle (7). While no explicit experiments have been undertaken to determine if phosphorylation has an effect on the physiological properties of this muscle, one report does imply that an effect does exist. Kushmerick and Paul have measured the total isometric energy use as a function of the tension-time integral (67). Their results indicate that, after an initial contraction period, the energy rate becomes steady. During this initial contraction time, the rate of hydrolysis was higher as inferred from the statistically significant greater than zero y-intercept on a plot of the total energy use versus tension-time integral. This intercept indicates an
initially rapid rate of energy use that decreases and becomes steady after a few seconds of contraction.

The high ATPase rate may be associated with the nonphosphorylated cross-bridges and the lower rate with phosphorylated cross-bridges. Phosphorylation can then be viewed as an energy-conserving mechanism invoked during long sustained contractions. Although less pronounced, this phenomenon appears identical to the effect in the EDL muscle and suggests that a series of experiments be undertaken to study this mechanism in frog muscle.

Myosin Heavy Chain Isozyme Composition

Reiser and co-workers (103) have reported that the maximum shortening velocity in individual rabbit soleus fibers is quite variable. The effects were found to correlate with the myosin heavy chain isozyme content but, somewhat surprisingly, not with the light chain content. There data are shown in Table 6 (Greaser, M. L., personal communication, Muscle Biology Laboratory, University of Wisconsin, March 1986).

By considering the velocity differences to be due to the myosin isozyme changes, the thermodynamic model for the unloaded contraction velocity can be derived as

\[
\frac{V_M}{V_M(x^F = 0)} = 1 + \frac{x^F(\alpha - 1)}{\beta + x^F(1 - \beta)}
\]

where the relative conductance terms are defined by
TABLE 6. Unloaded shortening speeds and isozyme content

<table>
<thead>
<tr>
<th>Isozyme Fraction</th>
<th>Unloaded Velocity of Shortening $V_f$</th>
<th>Normalized Unloaded Velocity of Contraction $V_f/V_m$ ($x^f = 0$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00</td>
<td>0.77</td>
<td>1.00</td>
</tr>
<tr>
<td>0.37</td>
<td>1.33</td>
<td>1.73</td>
</tr>
<tr>
<td>0.62</td>
<td>1.77</td>
<td>2.30</td>
</tr>
<tr>
<td>0.86</td>
<td>2.02</td>
<td>2.62</td>
</tr>
<tr>
<td>0.87</td>
<td>2.46</td>
<td>3.19</td>
</tr>
<tr>
<td>0.93</td>
<td>2.25</td>
<td>2.92</td>
</tr>
<tr>
<td>0.98</td>
<td>2.99</td>
<td>3.88</td>
</tr>
</tbody>
</table>

In these equations the superscripts $f$ and $s$ represent the 'fast' and 'slow' types of myosin heavy chains, respectively. By using nonlinear regression, the straight conductance term was found to be 2.11 with a standard error of 0.90. While the authors have suggested a single parameter linear model fits the data ($\beta = 1$), the presence of the second coefficient reduces the sum of squares error by 30 percent. Unfortunately, the straight conductance term is not significantly different from one ($P > .05$). This appears to be due to the small number of points when compared to the scatter in the data, and further
experiments are needed to clarify this point. The model parameter estimates and statistics are given in Table 9, and the model is plotted in Figure 22.

TABLE 7. Comparison of maximum contraction velocity models

<table>
<thead>
<tr>
<th>Model</th>
<th>Parameter Values</th>
<th>Sum of Squared Residuals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linear</td>
<td>$\alpha = 3.34$ (0.17)</td>
<td>0.632</td>
</tr>
<tr>
<td>Nonlinear</td>
<td>$\alpha = 3.64$ (0.27), $\beta = 2.11$ (0.90)</td>
<td>0.448</td>
</tr>
</tbody>
</table>

$^a$Parentheses indicate standard errors.

Unfortunately, changes in the isometric rate of energy use have not been investigated with these fibers. This point should be studied since the theory predicts substantial variations with changes in isozyme type. This variation can be estimated as

$$\frac{v_o/P_o(\kappa^f = 1)}{v_o/P_o(\kappa^f = 0)} \approx 3.6$$

assuming that the coupling coefficients of the two types of cross-bridges are equal.
FIGURE 22. Unloaded shortening velocity versus myosin isozyme content
SUMMARY

The theory of nonequilibrium thermodynamics is used to investigate the behavior of systems of energy converters. Relationships are developed that describe how system properties change with changes in the phenomenological (kinetic) properties of the subunits that make up the system. Two different subunit assemblies are considered (referred to as parallel-input parallel-output and parallel-input series-output) -- each containing two phenomenologically distinct types of subunits. Changes in various properties including limiting operating states, the energy flow needed to support these states, and the system coupling are all described as functions of the types of subunits within the system. Some properties are shown to vary in a linear manner with subunit changes and the input flows supporting these states also vary linearly. If nonlinear variations do occur, they are associated with internal flow or force cycles within the system. The changes for the input flows supporting these states can be linear or nonlinear, and extreme points are possible. A system coupling coefficient can also be defined and can exhibit a minimum, but it is never possible for a maximum to occur. More complex situations are briefly discussed.

The theory is applied to muscle contraction, which can be considered as a parallel-series type of system. Because of the unknown number of active cross-bridges, not all of the relationships derived for the parallel-series system can be used. Only properties that are not functions of the number of active cross-bridges are investigated -- these include the maximum velocity of contraction, isometric rate of
energy use normalized to the tension, and the system coupling. The theory is applied to the phosphorylation data of Crow and Kushmerick (33,34,35), Kushmerick and Crow (66), Barsotti and Butler (11), and Butler and co-workers (19); and to the isoenzyme data of Reiser and co-workers (103).
CONCLUSIONS

The basic conclusions of this work can be summarized as follows:

1. Overall or system phenomenological equations can be derived for systems composed of independent energy converters. These equations remain linear in respect to the system flows and forces, but each phenomenological term becomes considerably more complex. The form of these terms depends on the organization of the subunits in the system.

2. Models of this type show that even for systems composed of linear subunits, nonlinear output behavior can be produced in a nondissipative manner. This character develops when the number or types of subunits within the system are regulated.

3. For systems composed of many types of subunits but with only a single subunit being varied, the complex model that results is functionally identical to the simpler two-subunit type model. The functional constitutive coefficients in this simpler model all depend on the values of the phenomenological coefficients and the corresponding fractions of the unvaried subunits in the complex model. This enables the model that describes a two-subunit type system to be used in more complicated situations.

4. An overall coupling coefficient can be defined which describes the magnitude of the overall cross-phenomenological effect relative to the conjugated force-flow effects. This parameter differs from the individual coupling coefficient in that the highest level of coupling
possible corresponds to the coupling of the greatest coupled subunit. System coupling can be less than the coupling of the least tightly coupled subunit.

5. The subunit-system theory is able to account for and predict changes in mechanical and energetic properties of muscle have been previously related to biochemical variations found in the cross-bridge subunits. Decreases in the maximum velocity of contraction and the rate of energy use to tension ratio during an isometric contraction may be quantitatively described by the theory.

6. The subunit-system theory predicts high degrees of coupling and maximum efficiency in muscle that are consistent with experimental observations.
There are several areas, both specific and general, where continued work would be beneficial.

1. Specific to muscular contraction, an assumption was made that the fractional representation of "active" cross-bridge types is equal to the total fractional representation of cross-bridges. While this equality is implicitly assumed in all reported experimental data and is necessarily true in the two limits of cross-bridge types, no evidence exists to substantiate this assumption. Since phosphorylation or Ca$^{2+}$ binding would occur in a spatially random pattern there would be no physical limitation to the equal representation of cross-bridge types. The question then becomes one of whether there is an unequal kinetic probability of binding or resetting for the different types of cross-bridges, and experiments should be devised to test this.

2. Work should continue to find what additional regularities in the general theory may exist—especially in more complex systems. For example, as was noted for the three subunit type system, a topographical representation of some properties can be linear. Since linearity can greatly reduce the data needed to describe these surfaces, further work should be carried out to determine when linear relationships are to be expected.

3. As was pointed out by Caplan, some active transport systems appear to behave in a linear fashion, and hormones have been found to affect
the properties of some of these systems (see 25). A study should be undertaken to test if active transport behaves as a linear parallel-parallel system. It should be noted that, as in muscle, extracellular active transport has certain complicating factors in that an additional passive series resistance and a passive parallel leak may be present. Yet, even with these additional factors, the system equations may still be linear as Essig and Caplan have shown (43).

4. Finally, consideration is given here only to parallel-input systems. While most of the biological applications fall into this class, other systems may exist where the input behaves in a series manner. The mitochondria electron transport chain is an example. Electrons flow in this system through a series of cytochromes resulting in the pumping of protons across the inner mitochondrial membrane. Since at steady state the electron flow must be a constant through all of the elements in the chain, the system is assembled with the input flow in series. Research should be started to describe the nature of these energy conversion processes and to determine if areas of application exist.


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91. Pemrick, S. M. Is it possible for a single modifier to exert both a positive and a negative effect on the skeletal actomyosin MgATPase? Biophys. J. 47: 62a; 1985.


124


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